

Open Research Online

The Open University's repository of research publications and other research outputs

The Role of HPV E7 in Maintenance of Cell Transformation

Thesis

How to cite:

Basukala, Om (2019). The Role of HPV E7 in Maintenance of Cell Transformation. PhD thesis The Open University.

For guidance on citations see [FAQs](#).

© 2019 The Author



<https://creativecommons.org/licenses/by-nc-nd/4.0/>

Version: Version of Record

Link(s) to article on publisher's website:

<http://dx.doi.org/doi:10.21954/ou.ro.00010bfd>

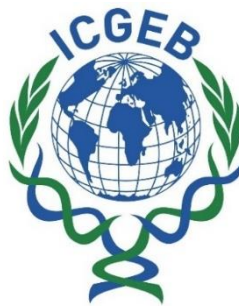
Copyright and Moral Rights for the articles on this site are retained by the individual authors and/or other copyright owners. For more information on Open Research Online's data [policy](#) on reuse of materials please consult the policies page.

oro.open.ac.uk

The role of HPV E7 in maintenance of cell transformation

Om Basukala

A thesis submitted to The Open University for the degree of Doctor of Philosophy in the School of Life, Health and Chemical Sciences



**International Centre for Genetic Engineering and
Biotechnology (ICGEB),**

Trieste, Italy

Director of Studies: Lawrence Banks, PhD

Second Supervisor: John Doorbar, PhD

October 2019

Acknowledgement

I am very much thankful to my supervisor, Lawrence Banks, for all time support, guidance and encouragement. This thesis would not have been possible without his excellent guidance, supervision and support. Working and learning in Tumour Virology Lab has been an amazing time for me for all these years. Thank you very much for this extraordinary opportunity.

I am grateful to John Doorbar, my second supervisor for excellent ideas, critical analysis, brainstorming and relentless discussion sessions for our queries.

I am equally thankful to all the members of Tumor Virology Lab. Miranda Thomas for training me in laboratory skills and correcting the manuscripts. Paola Massimi for excellent support with experiments. David Pim for insightful discussions and funny remarks. Suruchi Mittal for guidance with CRISPR experiments. All current and past fellow colleagues from the lab - Justyna Broniarczyk, Marina Paula Bugnon Valdano, Maria Paula Dizanzo, Mona Elsherbeny, Emily Montosa Nunes, Carla Vanessa Sarabia Vega, Abida Siddiq, Neva Skrabar, Anita Szalmas, Jayashree Vijay Thatte, Vjekoslav Tomaic, Oscar Trejo, Arushi Vats, and Amira Zine El Abidine – you guys are amazing! Thank you all for chats, laughs, coffees and drinks, movies and trips to places.

I am thankful to Mike P. Myers and Corrado Guarnaccia for proteomics facility and analysis and Marco Bestagno for FACS analysis.

I am thankful to my tutor, Vittorio Venturi for being there in need of support. I am equally grateful to Federica Benvenuti, Franco Pagani and Francesco Loffredo for progress monitoring over several checkpoint analyses during my studies.

I really appreciate and am very much thankful to the meetings and courses unit, fellowship unit, procurement unit, IT and library services and all ICGEB units for all time support in ICGEB over the years of my studies.

To my parents and family for all the support, love and care - lots of love to you all!

October 2019, Trieste, Italy

Abstract

A hallmark feature of cervical cancers caused by the Human Papillomavirus is the continued and high-level expression of viral oncoproteins. These viral oncoproteins play a significant role in induction of malignancy by targeting several critical cell control pathways. One of the potentially important elements in this process is phosphorylation of E7 by Casein Kinase II (CKII) which induces S-phase entry and modulates association with pRB and other pocket proteins. Phosphorylation of E7 has also been known to enhance E7's ability to interact with other cellular targets and thereby increasing the ability of E7 to enhance cell proliferation and induce malignancy. However, there is little information on the importance of this site in E7, once the tumour cells have become fully transformed. To determine this, we have generated genome edited cell lines at CKII phospho-acceptor site of HPV-18 E7 in C4-1 cervical tumour derived cells using CRISPR/Cas9. We show that the HPV-18 E7 S32A/S34A mutation abolishes CKII phosphorylation. Genome edited cells with this mutation continue to proliferate but are more slow-growing than wild type cells, reach lower saturation densities and are susceptible to low nutrient conditions. The invasive ability of these cells is markedly reduced, partly due to downregulation of matrix metalloproteases (MMPs). Mechanistically, we find that phosphorylation of E7 plays a direct role in the ability of E7 to activate AKT signaling, which in turn is required for optimal levels of MMP secretion. Using proteomic analysis, we identify Vangl1, planar cell polarity protein, as a novel CKII phospho-dependent interactor of E7. The steady levels of Vangl1 are downregulated in genome edited cells and it co-operates with HPV-16 E7 in BRK cell transformation assays, indicating Vangl1 being important for E7 transforming activity. Finally, I also show that HPV-E7 interacts with PTPN21, a non-receptor tyrosine phosphatase, via c-terminal region of E7. The interaction leads to destabilization of PTPN21 by high-risk E7, suggesting a potential role of E7 in affecting post-translation modifications of its targets. Thus, HPV E7 continue to play an important role in maintenance of transformed phenotype and targeting E7 phosphorylation could be a potential therapeutic strategy in HPV-induced malignancy.

List of Figures

- Figure 1. The hallmarks of cancer adapted from (Hanahan and Weinberg, 2016).
- Figure 2. Organization of the HPV-16 genome, adapted from (Doorbar, 2006).
- Figure 3. Progression from a productive HPV infection to malignancy, adapted from (Thomas et al., 2008).
- Figure 4. Schematic representation of HPV-16 E6.
- Figure 5. Schematic representation of HPV-16 E7.
- Figure 6. Hypothetical model of HPV-E7 showing various cellular binding partners.
- Figure 7. Hypothetical model of HPV-16 E7/Cullin 2 complex.
- Figure 8. Schematic representation of the known HPV E7 post translational modifications.
- Figure 9. The E7 LXCXE binds as an extended peptide onto a conserved groove of B-box of RB (Lee et al., 1998).
- Figure 10. Post translational modification of HPV E7 by ubiquitin proteasome pathway.
- Figure 11. Wnt/PCP signaling in cancer cell migration.
- Figure 12. Diagrammatic representation of Vangl1 protein.
- Figure 13. Schemantic representation of domains in PTPN14 and PTPN21.
- Figure 14. Mutation of HPV-18 E7 wild type to S32A/S34A abolishes phosphorylation by casein kinase II (CKII).
- Figure 15. C4-1 cells are dependent on continued expression of E6 and E7 for growth and proliferation.
- Figure 16. Design and efficiency analysis of gRNAs targeting CKII site in HPV-18 E7 in C4-1 cells.
- Figure 17. Schematic representation of strategic protocol for CRISPR/Cas9 genome editing HPV-18 E7 S32A/S34A in C4-1 cells.

- Figure 18. Design of HDR repair template for genome editing CKII site in HPV-18 E7 and screening and validation of edited C4-1 clones.
- Figure 19. Growth of CKII site mutant cells is reduced in low nutrient conditions.
- Figure 20. Steady-state levels of HPV-18 E7 and pocket proteins in the C4-1 cell lines.
- Figure 21. CKII site mutant C4-1 cells show defects in matrigel invasion.
- Figure 22. Scratch wound healing assay for migratory abilities of C4-1 cells.
- Figure 23. Defect in ability to hydrolyse gelatin linked to downregulation of MMP13 in CKII site mutant C4-1 cell lines.
- Figure 24. Matrix metalloproteases MMP 1 and MMP 13 are downregulated in CKII site mutant C4-1 cell lines.
- Figure 25. Higher levels of MMP1 and MMP13 in condition medium of C4-1 wild type is independent of cell proliferation.
- Figure 26. Matrix metalloproteases MMP 1 and MMP 13 are downregulated in CaSki but not in C33a cells after CKII inhibition.
- Figure 27. Exogenous expression of wild type HPV-18 E7 can rescue the downregulation of MMP 1 and MMP 13 in CKII site mutant cell lines.
- Figure 28. Stable expression of wild type HPV-18 E7 can rescue the downregulation of MMP 1 and is dependent on intact phospho-acceptor site in E7.
- Figure 29. Knockdown of MMP1/13 abrogates invasion of C4-1 and CaSki cells.
- Figure 30. Validation of HPV-16 E7 phospho-specific antibody.
- Figure 31. Activation of AKT is perturbed in the absence of CKII phosphorylation of E7.
- Figure 32. Inhibition of PI3/AKT pathway downregulates MMP1/13 in HPV-positive, C4-1 and CaSki cells but not in HPV-negative C33a cells.

- Figure 33. Biotinylated peptide sequences used for pulldown assay and downstream proteomic analysis.
- Figure 34. Validation of Vangl1 as phospho-specific interactor of HPV-16 E7 phospho-peptide.
- Figure 35. HPV-16 E7 interacts with Vangl1 *in vivo* and is dependent on phosphorylation of E7.
- Figure 36. Vangl1 interaction with HPV-16 E7 is perturbed in presence of specific CKII inhibitor.
- Figure 37. Vangl1 interaction with high- and low-risk E7s in a CKII phosphorylation dependent manner.
- Figure 38. Vangl1-phospho E7 interaction is perturbed in genome edited C4-1 cells.
- Figure 39. Vangl1 enhances BRK cell transformation ability of E7 in HPV-16 E7/EJ-ras transformation experiments.
- Figure 40. Steady state levels of MMP14 in C4-1 cell lines.
- Figure 41. PTPN21 interacts with high-risk and low-risk HPV-E7s.
- Figure 42. Identification of residues in CR3 region of E7 responsible for binding with PTPN21.
- Figure 43. Lower levels of PTPN21 protein in HPV-positive cells is due to its destabilization via proteasome-mediated degradation.
- Figure 44. Downregulation of E7 in HPV positive cell lines rescues levels of PTPN21.
- Figure 45. PTPN21 is a degradation target of high-risk HPV E7s.
- Figure 46. Generation of phospho-specific antibodies against HPV-16 E7 S31S32.
- Figure 47. Vangl1 expression and mutation profile across several cancer data sets accessed via cBioPortal.
- Figure 48. p53 expression and mutation profile across several cancer data sets accessed via cBioPortal.

List of Tables

Table 1. Identification of phospho-specific interactors of HPV-16 E7.

Publications

Basukala, O., Mittal, S., Massimi, P., Bestagno, M., & Banks, L. (2019). The HPV-18 E7 CKII phospho acceptor site is required for maintaining the transformed phenotype of cervical tumour-derived cells. PLoS Pathog, 15(5), e1007769. doi:10.1371/journal.ppat.1007769

Szalmas, A., Tomaic, V., Basukala, O., Massimi, P., Mittal, S., Konya, J., & Banks, L. (2017). The PTPN14 Tumor Suppressor Is a Degradation Target of Human Papillomavirus E7. J Virol, 91(7). doi:10.1128/JVI.00057-17

Abstract	i
Acknowledgements	ii
List of Figures	iii
List of Tables	vi
Publications	vii

Contents

Introduction	1
Hallmarks and etiology of cancer	1
HPV infection and HPV attributable cancer	3
Molecular Biology of HPV	5
Virus structure and genome organization	5
Viral life cycle	6
Early proteins – E1 and E2	6
Oncoproteins – E6 and E7	7
Early proteins – E4 and E5	8
Viral packaging and release	8
HPV E6 and E7 oncoproteins	9
E6 oncoprotein	9
Role of HPV E6 in cell transformation	10
Inactivation of p53 tumour suppressor and abrogation of apoptotic signaling	10
Role of E6 PDZ binding motif (PBM)	12
Modulation of cellular signaling pathways	13
Telomerase activation	14
E7 oncoprotein	16
Role of HPV E7 in cell transformation	17
Association of E7 with pRb and activation of E2F-dependent transcription	19

G1/S cell checkpoint deregulation by other mechanisms	21
HPV E7, senescence and autophagy	22
E7 and genomic instability	23
HPV E7 and Epigenetic reprogramming	24
Modulation of micro RNAs by HPV E7	26
UBR4/p600, Anoikis and anchorage independence	27
PTMs of HPV E7 and their role in HPV biology and carcinogenesis	28
Phosphorylation	28
Ubiquitination of E7	33
Other modifications	35
Polyamination of HPV 18E7	35
Therapeutic implications of studying E7 PTMs	36
CKII and CKII inhibitors in HPV-associated cancers	37
MMPs and HPV mediated carcinogenesis	40
Novel targets of HPV E7	42
Wnt/PCP (Planar cell polarity) and cancer	42
Van Gogh-like proteins - Vangl1 and Vangl2	45
Role of Vangl1 in cancer	46
Non-receptor protein tyrosine phosphatases - PTPN14 and PTPN21	47
PTPN21	48
Thesis Aims	50
Results	51
Section I	
The HPV-18 E7 CKII phospho acceptor site is required for maintaining the transformed phenotype of cervical tumour-derived cells	51
Mutation of HPV-18 E7 wild type to S32A/S34A abolishes phosphorylation by casein kinase II (CKII)	51

C4-1 cells are dependent on continued expression of E6 and E7 for growth and proliferation	52
Genome editing E7 wild type to S32A/S34A in C4-1 cells	53
CKII site mutant cells are less proliferative in low nutrient conditions	56
Levels of pocket proteins in CKII mutant cells and CKII phospho-acceptor site dependent interaction of E7 with pRB	57
CKII mutant cells are less invasive in Matrigel invasion assay	59
Defective invasive ability of CKII mutant cells links to downregulation of MMPs	61
Downregulation of MMP1/13 can be rescued in CKII mutant cells by exogenous expression of wild type HPV-18 E7	64
Downregulation of MMP1 and MMP13 in C4-1 and CaSki cells downregulates invasive ability	67
CKII phospho-acceptor site in high-risk E7 is important for sustained activation of AKT and expression of extracellular MMP1/13	69
Section II	
Planar cell polarity protein Vangl1 is targeted by Human Papillomavirus E7 oncoprotein	72
Proteomic analysis identifies Vangl1 as novel interactor of HPV-16 phospho-E7	72
Interaction of Vangl1 with phospho-E7 is conserved among high-risk and low-risk types	78
Vangl1-phospho E7 interaction is perturbed in genome edited C4-1 cells	79
Vangl1 co-operates with HPV-16 E7 in cell transformation	80

Section III	
PTPN21 is a degradation target of high-risk HPV E7	83
PTPN21 interacts with HPV E7s	83
PTPN21 is a degradation target of high-risk E7	85
Discussion	89
Section I	
The HPV-18 E7 CKII phospho acceptor site is required for maintaining the transformed phenotype of cervical tumour-derived cells	89
Section II	
Planar cell polarity protein Vangl1 is targeted by Human Papillomavirus E7 oncoprotein	96
Section III	
PTPN21 is a degradation target of high-risk HPV E7	99
Materials and methods	103
Appendix I	112
Appendix II	114
Appendix III	128
References	130

Introduction

Hallmarks and etiology of Cancer

The human body is composed of many different types of cells which are the basic building blocks that make up the body. These cells possess specific characteristics that help maintain functioning of tissues, organs and organ systems. Normal cells maintain normal cell proliferation or controlled growth, undergo programmed cell death in response to unrepairable damage and maintain tissue homeostasis, differentiate into specialized cells with a specific function and maintain selective adhesions ensuring to remain in their intended location. However, due to specific changes in the genes of one or a group of cells, they can evade the normal cell characteristics affecting their growth and division, leading to development of a tumour and often acquire potential to invade and/or spread to different locations in the body.

The hallmark features of these deregulated or cancer cells are acquired over the process of tumor development and malignant progression. Cancer cells in general are thus able in sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, deregulating cellular energetics and metabolism, and avoiding immune destruction (Hanahan and Weinberg, 2000, 2011). The acquisition of these eight functional capabilities are primarily facilitated by two main traits of cancer – genome instability with consequent gene mutation and tumor-promoting inflammation. All these diverse capabilities are brought about by heterotypic interactions between different cell types in the tumour microenvironment, composed of cancer cells and tumour-associated stroma which includes recruited support cells (angiogenic vascular cells, fibroblasts and infiltrating immune cells). Thus, cancer cells are continually undergoing genetic diversification during tumour progression assuming a variety of distinctive phenotype and pathogenesis of diverse forms of human cancer (Hanahan and Weinberg, 2011, 2016) (Figure 1).

The major cause of most human cancers is exposure to agents that cause DNA damage and genomic instability leading to attaining above mentioned hallmarks of cancer over the period of tumour development and malignant progression.

Smoking, radiation, cancer-causing chemicals or carcinogens, infectious agents (viruses and bacteria), obesity, hormones, chronic inflammation and lack of exercise have been linked to play a role in causing cancer (Cogliano et al., 2011; de Martel et al., 2012). Among others, infectious agents like hepatitis B virus (HBV), hepatitis C virus (HCV), Human Papillomavirus (HPV), Epstein Barr Virus (EBV), HIV-1, human T-cell lymphotropic virus-1, Merkel Cell Polyomavirus (MCV), Kaposi's sarcoma herpesvirus (KSHV), *Helicobacter pylori*, *Schistosoma hematobium*, and *Opisthorchiasis viverrini* are known to cause approximately 15% of human cancers (de Martel et al., 2012; Plummer et al., 2016). Most viruses have evolved to use host cellular gene products for their life cycle, however, certain tumour viruses such as HPVs are able to express specific viral genes (oncogene) that regulate proliferative or anti-apoptotic activities through interaction with cellular gene products and can directly contribute to cancer hallmarks (Moore and Chang, 2010; Schiller and Lowy, 2014).

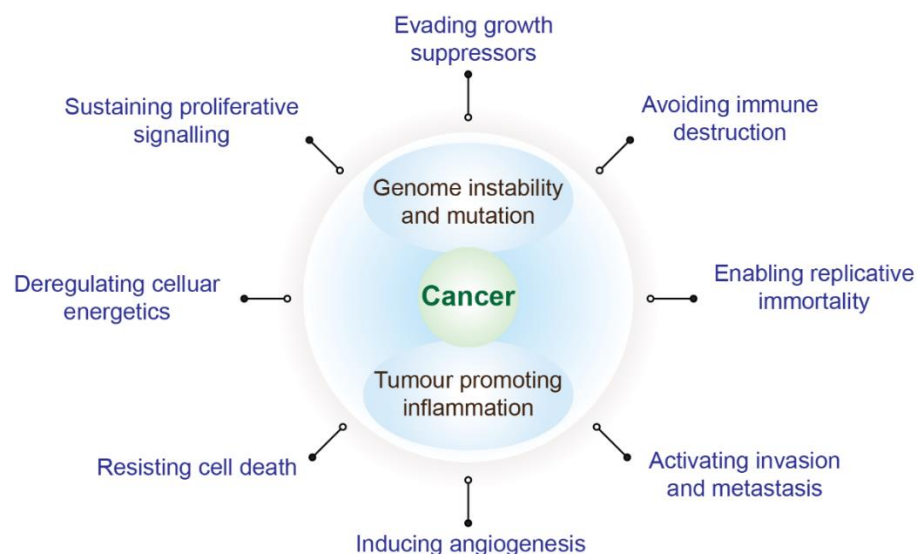


Figure 1. The hallmarks of cancer adapted from (Hanahan and Weinberg, 2016).

A schematic illustrating eight distinct functional capabilities (radial bullets) and two facilitators (bullets in oval bubble), that are necessary conditions to manifest malignant disease - Cancer.

HPV infection and HPV attributable cancer

Cervical cancer is the 2nd most common female cancer in women aged 15-44 years and ranks the 4th leading cause of female cancer worldwide, with an estimated 527,624 new cases and 265,672 deaths (Bruni L, 2017; GLOBOCAN, 2012). Almost one third of the 15.4% human cancers attributable to carcinogenic infections are caused by human papillomaviruses (HPVs) (Plummer et al., 2016). Cervical cancer and a significant number of other anogenital cancers, plus a rapidly increasing number of head and neck cancers, including those in the oral cavity, oropharynx, sinus, tonsil and larynx (Cutts et al., 2007; Moody and Laimins, 2010) are caused by these viruses. In the case of cervical malignancy this is almost always associated with infection with HPVs. The WHO has recently defined at least 12 different HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 – collectively known as high-risk types) as being cancer-causing in the cervix, and cervical cancer is one of the devastating causes of cancer mortality in the developing parts of the world (Cutts et al., 2007; de Martel et al., 2017; UK., 2012; WHO, 2017a).

HPVs belong to the Papillomaviridae family. They are non-enveloped virions containing a double-stranded DNA genome enclosed by icosahedral capsid composed of major (L1) and minor (L2) structural proteins. These viruses infect cutaneous and mucosal epithelium. To date, over 200 HPV types have been identified based on the genomic sequence of the principal capsid protein (L1). The HPV types can be classified as cutaneous or mucosal based on their potential infection site in the body and by their potential to induce malignancy as high-risk and low-risk types.

Infection with HPV is believed to occur through contact with infected genital skin, mucous membranes or bodily fluids and can be sexually transmitted. Most (70-90%) of these infections are asymptomatic and resolved by the host immune response within 1-2 years. While in some (5-10% of infected) individuals, where the virus is not cleared by the immune system, this can lead to a persistent infection which is one of the biggest risk factors for progression to invasive carcinoma (Doorbar et al., 2012). Persistent infection is a necessary cause of cervical cancer and is defined by the presence of type-specific HPV DNA on

repeated clinical biological samples over a period-of-time (usually >6 months). Such persistent infection may progress to premalignant glandular or squamous intra-epithelial lesions, histopathologically classified as cervical intra-epithelial neoplasia (CIN) and to cancer (Bosch et al., 2002; WHO, 2017b).

Diagnosis of cervical HPV infection can be done using tests based on identification of HPV DNA from cervical or vaginal swabs. While changes in the cervical epithelium due to HPV can be detected by Papanicolaou (Pap) test – a microscopic examination of exfoliated cells. In low-resource settings, visual inspection with acetic acid are used to identify lesions and screen for cervical cancer (WHO, 2013b).

Virus-specific treatment of HPV infection is not available, while screening and treatment for pre-invasive disease of the cervix is widely successful in preventing progression to cervical cancer. Such methods of treatment include removal or destruction of the abnormal tissue by burning or freezing (cryotherapy) and/or surgical removal (loop electrosurgical excision procedure [LEEP] or cone biopsy) (WHO, 2013a).

HPV-attributable cancers can be prevented by vaccines and cervical cancer is one of the most preventable cancers. Currently, three prophylactic vaccines are available – (i) Cervarix (GlaxoSmithKline), (ii) Gardasil (Merck & Co.) and (iii) Gardasil 9 (Merck & Co.). All three vaccines protect against infection with high risk HPV type 16 and 18 associated with 73% of cervical cancers (de Martel et al., 2017). In addition, Gardasil protects against low-risk HPV type 6 and 11 and Gardasil 9 protects against next five most carcinogenic types (HPV 31, 33, 45, 52, 58) in addition to HPV 16, 18, 6, 11; all together causing 90% HPV-attributable cancers (de Martel et al., 2017; WHO, 2017b).

Although these vaccines have the potential to have a major impact on the global burden of HPV attributable cancers, they are purely prophylactic and have no therapeutic potential. Therefore, many women that are infected with the virus will still develop cervical cancer. In addition, accessibility in terms of both cost and logistics in many developing countries, is a major issue (reviewed in (Roden and Stern, 2018)). Thus there is still a pressing need to develop better forms of therapeutic interventions (Manzo-Merino et al., 2013) and understanding the

mechanisms by which these viruses bring about malignancy, thus offers, one potential means of developing novel intervention strategies.

Molecular Biology of HPV

Virus structure and genome organization

HPV are small, icosahedral non-enveloped, 50-60 nm in diameter and enclose a circular, double-stranded DNA genome of approximately 8 kb. Each capsid is made up of 360 molecules of L1 protein arranged into 72 capsomeres and around 72 copies of L2 protein (Buck et al., 2008; Sapp et al., 1995). The genome contains eight or nine ORFs designated early (E1, E2, E4, E5, E6, and E7) or late (L1 and L2) depending on the time of expression after infection. The expression of these ORFs is regulated by multiple promoters and a complex pattern of splicing. The genome also includes a long control region (LCR) or upstream regulatory region (URR), which contains cis-responsive elements responsible for controlling viral replication and gene expression. A schematic depiction of HPV-16 genome organization is shown in Figure 2.

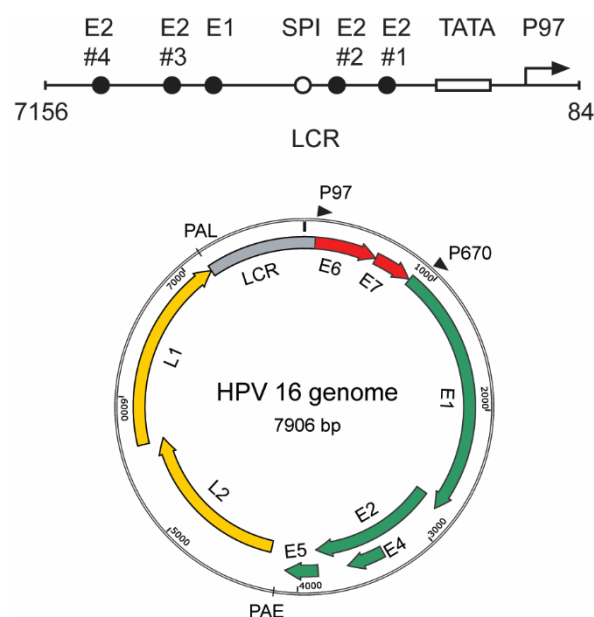


Figure 2. Organization of the HPV-16 genome, adapted from (Doorbar, 2006).

A. The graphical representation of 7906 bp of HPV16 genome is shown with the early (p97) and late (p670) promoters marked by arrows. The six early ORFs [E1, E2, E4 and E5 and E6 and E7] are expressed from either p97 or p670 at different stages during epithelial cell differentiation. The late ORFs [L1 and L2] are also expressed from p670, following a change in splicing patterns, and a shift in polyadenylation site usage [from early polyadenylation site (PAE) to late polyadenylation

site (PAL)]. All the viral genes are encoded on one strand of the double-stranded circular DNA genome. The long control region (LCR from 7156–7184) is enlarged to allow visualization of the E2-binding sites and the TATA element of the p97 promoter.

Viral life cycle

The life cycle of HPVs is closely linked to the differentiation profile of the host epithelium. It is believed that the initial infection requires access to the cells in the basal layer, through micro-traumas in the skin. The virus particle requires cell surface receptors heparin sulphate proteoglycans and possibly laminin for attachment. The virus is then suggested to be internalized with the help of $\alpha 6$ integrin and growth factor receptors, however, the exact identity of the receptor for entry is controversial (Culp et al., 2006; Evander et al., 1997; Giroglou et al., 2001; Johnson et al., 2009). After internalization, virions undergo endosomal transport, uncoating and cellular sorting; ensuring nuclear entry of L2-DNA complex while leaving L1 protein for lysosomal degradation (Bergant Marusic et al., 2012; Schelhaas et al., 2012). In the basal layer, the viral genome is then maintained in a low copy episome (Pyeon et al., 2009; Schiffman et al., 2016). The infected basal cells divide and eventually undergo cellular differentiation program towards the parabasal layer. In case of a productive life cycle (Figure 3), as a result of co-ordinate expression of viral proteins (E1, E2, E4, E5, E6, E7, L1 and L2) with ongoing differentiation program of the infected cell, viral genome amplification then typically occur in the mid-epithelial layers and virus assembly and release at the epithelial surface giving rise to new infectious viral particles (Schiffman et al., 2016).

Early proteins – E1 and E2

The viral proteins E1 and E2 play important role in initial amplification phase. E1 protein has ATPase and helicase activity essential for viral DNA replication (Sakakibara et al., 2011). E2 is a DNA-binding protein. It regulates viral transcription and has multiple binding sites in the viral LCR (long control region). It recruits the viral E1 helicase to the viral origin of replication, which in-turn is involved in replicating the viral genome by recruiting host replication proteins (McBride, 2008). E2 protein can also function as transcriptional repressor for the expression of viral oncoproteins E6 and E7 (Dowhanick et al., 1995; Thierry and Yaniv, 1987).

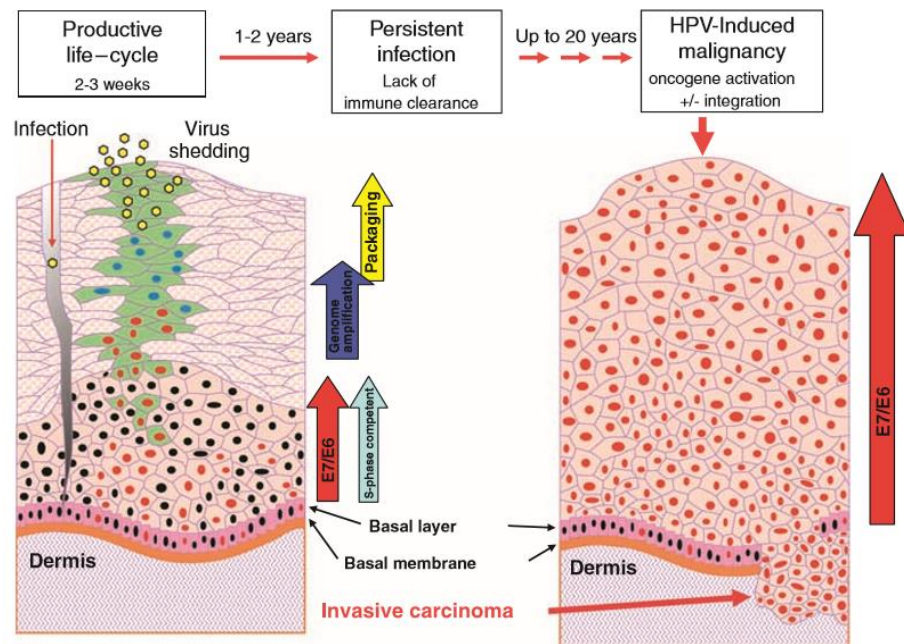


Figure 3. Progression from a productive HPV infection to malignancy, adapted from (Thomas et al., 2008).

Infection with HPV is usually acquired through micro-traumas in the skin infecting the epithelial mucosa basal cells. As these cells divide, there is coordinate expression of different viral gene products with E6/E7 causing differentiated cells back into S-phase competent cells. This allows viral genome amplification and ultimately, the synthesis and shedding of new viral particles within a period of 2-3 weeks. In the case of persistent infection, lesions are not resolved, and high levels of viral DNA can be detected over extended periods of time. This ultimately pre-disposes the host to the development of a malignancy. This is characterized by a loss of differentiation, no viral replication, and high levels of E6 and E7 oncoprotein expression.

Oncoproteins – E6 and E7

A clear role for low risk E6 and E7 proteins in infected basal cells is uncertain, while in the case of high-risk types, it has been shown that E6 and E7 drive cell proliferation in the basal and parabasal cell layers (Barrow-Laing et al., 2010; Zhang et al., 2006). The coordinate expression of E6 and E7 proteins create an environment favorable for viral DNA replication, achieved primarily through interfering with cellular growth control pathways, with E7 targeting many elements involved in the control cell of cycle, whilst E6 inhibits the pro-apoptotic response of the cell to this unscheduled DNA replication. The molecular details of how these oncoproteins do this will be discussed later. Despite functional

differences between high and low risk HPV type, the main function of E6 and E7 protein of most HPV types is to allow viral genome amplification by stimulating cell cycle re-entry occurring mainly in the mid-epithelial layers (reviewed in (Doorbar et al., 2012)).

Early proteins – E4 and E5

E4 proteins are expressed relatively late during infection before the expression of L2 and L1 and help in the maturation of viral particles. E4 proteins are highly expressed and facilitate efficient release and transmission of the new virions. High levels of E4 proteins are deposited as amyloid fibers and can be used as infection biomarkers (Doorbar, 2013). The E5 proteins are small hydrophobic proteins, localizing to endosomal membranes and the Golgi apparatus (Conrad et al., 1993). It is thought to augment oncogenic activities of E6 and E7 proteins, and allow the continuous proliferation of the host cell delaying differentiation (de Freitas et al., 2017; Venuti et al., 2011).

Viral packaging and release

HPV life cycle is ready to re-initiate with release of mature viral particles from superficial dying keratinocytes. This involves expression of L2, cell cycle exit and L1 to allow genome packaging (Doorbar, 2006). L2 proteins are recruited (by E2) to regions of replication to initiate encapsidation of the viral genome (Day et al., 1998; Holmgren et al., 2005). At this stage, transcripts that initiate at late promotor (p670 in HPV16) and terminate at late polyadenylation site are elevated, which is facilitated by higher levels of E2 which also down-regulate p97; finally leading to a change of E5 mRNA to a L1 mRNA (Doorbar, 2005; Johansson et al., 2012; Milligan et al., 2007; Ozbun and Meyers, 1998). Oxidizing environment at superficial dying keratinocytes then enables the progressive accumulation of bisulfide bonds between L1 proteins leading to virus maturation and production of stable infectious virions (Buck et al., 2005; Doorbar et al., 2015; Finnen et al., 2003).

HPV E6 and E7 oncoproteins

One of the key features of HPV-associated neoplasia is the disordered expression of viral gene products, leading to deregulation of the viral life cycle. The neoplastic phenotype of the disease is thought to be a function of underlying levels of expression of E6 and E7 oncoproteins. CIN1 lesions typically retain the ability to complete the viral life cycle and production of viral particles, having a lower level of cell proliferation in the basal and parabasal layers and phenotypically resembling flat warts (Middleton et al., 2003). While in the case of CIN2/3+, there is an elevated expression of E6 and E7, allowing the cell to accumulate genomic changes that can eventually contribute to cancer progression. The deregulated viral gene expression in these neoplasia (CIN2/3+) is also thought to facilitate the integration of viral episome into the host chromosome, further deregulating the expression of E6 and E7 (Doorbar et al., 2015). In fact, a major feature of HPV-attributable cancers is the requirement for continued expression of the viral E6 and E7 oncogenes for continued proliferation and survival (Butz et al., 2003; Yamato et al., 2008; Yoshinouchi et al., 2003). Neither E6 nor E7 has enzymatic functions, but by interacting with many different cellular proteins they modulate various normal cellular signaling functions to induce a proliferative state that can progress towards cell transformation. In the following sections I aim to summarize some of these key functions of E6 and E7 oncoproteins that contribute towards carcinogenesis.

E6 Oncoprotein

The HPV E6 proteins are approximately 150 amino acids in length and are zinc binding proteins with four CXXC motifs (Barbosa et al., 1989; Grossman and Laimins, 1989). A schematic structure of E6 is shown in Figure 4. E6 is transcribed either as full-length (E6) mRNA or as one of several possible truncated E6 mRNAs (E6*) based on a complex splicing pattern. The E6* transcripts described in HPV-16 are known as E6*I, E6*II, E6*III, E6^ΔE7, E6^ΔE7*I, E6^ΔE7*II, E6*IV, E6*V and E6*VI (Ajiro and Zheng, 2015; Doorbar et al., 1990; Smotkin and Wettstein, 1986; Tang et al., 2006), while the four E6* transcripts that have been described for HPV-18 are E6*I, E6*II, E6*III and E6^ΔE7 (Ajiro et al., 2016; Pim and Banks, 1999; Pim et al., 1997).

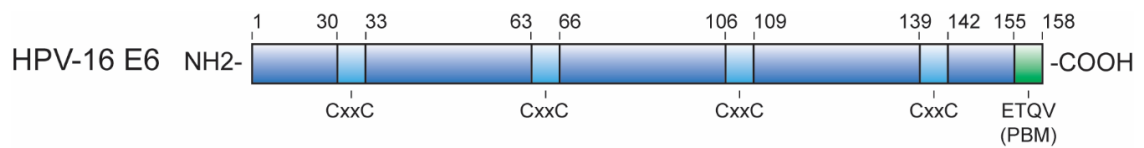


Figure 4. Schematic representation of HPV-16 E6.

The position of CXXC (Cys-X-X-Cys) motifs, which participate coordinating zinc ion are indicated. C-terminus PDZ binding motif (PBM) sequence 'ETQV' is shown, which can be phosphorylated by AKT, Chk2 via PKA and Chk1 kinases.

E6 is a multifunctional protein and interacts with numerous cellular targets: modulating inactivation of p53; blocking apoptosis; activating telomerase; disrupting cell adhesion, cell polarity and epithelial differentiation; altering transcription and reducing immune recognition (reviewed in (Howie et al., 2009; Vande Pol and Klingelutz, 2013)). The complete crystal structure of E6 including the N-terminal and C-terminal halves has been resolved and structure-functional analysis of E6 further suggests its plasticity in interacting with wide range of cellular substrates (Nomine et al., 2006; Thomas et al., 2016; Zanier et al., 2013; Zanier et al., 2012).

Role of HPV E6 in cell transformation

Inactivation of p53 tumour suppressor and abrogation of apoptotic signaling

One of the most well-studied interactions of E6 is with the p53 tumor suppressor (Werness et al., 1990). The p53 protein plays many roles in the cell, including cell-cycle regulation, activation of DNA repair pathways upon DNA damage and induction of apoptosis (Murray-Zmijewski et al., 2008). By interacting with p53, E6 checks the transcriptional functions of p53, leading to inhibition of p53-dependent gene expression (Mietz et al., 1992). In addition, high-risk E6 binds to an LXXLL motif on a cellular E3 ubiquitin-protein ligase, E6-associated protein (E6AP) (Brimer et al., 2007; Chen et al., 1998; Huibregtse et al., 1993b; Kuballa et al., 2007). The E6-E6AP complex then recruits and ubiquitinates p53, mediating its degradation via the proteasome (Huibregtse et al., 1991, 1993a; Scheffner et al., 1990). The E6-E6AP complex has also been shown to be important for E6 stability, and ablation of E6AP thus rescues p53 through two

routes: as result of E6 destabilization and the loss of E6AP ubiquitin ligase activity (Hengstermann et al., 2005; Tomaic et al., 2009).

E6 interaction with p53 has been shown to perturb binding of p53 to its site-specific DNA sequences (Lechner and Laimins, 1994), possibly as a result of conformational changes in the p53 protein upon interaction with E6, which, in turn, lead to an inhibition of p53 DNA binding ability (Thomas et al., 1995). Furthermore, E6 perturbs p53 function by sequestering p53 in the cytoplasm, potentially by sterically hindering the p53 nuclear localization signal (Mantovani and Banks, 1999). E6 has also been shown to abrogate transactivation of p53-responsive genes via interaction with CBP/p300, (Patel et al., 1999; Thomas and Chiang, 2005; Zimmermann et al., 1999), with hADA3 (Chand et al., 2014; Kumar et al., 2002; Sekaric et al., 2007), and by destabilizing TIP60 (Jha et al., 2010).

In addition, E6 can further abrogate apoptotic signaling by interacting with the pro-apoptotic protein Bak and mediating its degradation via the E6AP ubiquitin ligase (Thomas and Banks, 1998, 1999). E6 was also shown to inhibit differentiation-induced apoptosis in human foreskin keratinocytes by modulating the expression of anti-apoptotic Bcl-2 and pro-apoptotic Bax proteins (Alfandari et al., 1999). Further, inhibition of E6 was shown to result in the p53-dependent transcriptional activation of the PUMA promoter, leading to activation and translocation of Bax to the mitochondrial membrane, cytochrome c release into the cytosol and activation of caspase 3. In addition, inhibition of Bax expression in this context was shown to efficiently revert the apoptotic phenotype, suggesting that perturbation of the p53/PUMA/Bax cascade is an important antiapoptotic function of E6 in HPV-positive cancer cells (Vogt et al., 2006). Moreover, E6 interacts with the Fas-associated death domain (FADD) and procaspase 8 to enable cells to escape from Fas-triggered apoptosis (Filippova et al., 2007; Filippova et al., 2004). In addition, E6 has been shown to evade apoptosis by downregulating the pro-apoptotic transforming growth factor- β 2 (TGF- β 2) and thence downregulating TGF- β 2 responsive gene expression (Nees et al., 2000). The ultimate consequence of E6 targeting apoptotic pathways is to avoid apoptosis, which would otherwise eliminate the HPV-infected cell.

Role of E6 PDZ binding motif (PBM)

One of the unique features of high-risk E6 proteins is the presence of a PDZ (Post Synaptic Density 95 (PSD95), Discs Large (Dlg) and Zona Occludens 1 (ZO-1))-binding motif (PBM) (X-(S/T)-X-(V/I/L)-COOH), in the C-terminus, which is largely absent in E6 proteins of low-risk HPV types (Kiyono et al., 1997; Lee et al., 1997). The PBM is involved in binding to cellular proteins that have PDZ domains (Songyang et al., 1997). Some of the key PDZ domain-containing proteins that are bound by the E6 PBM are discs large tumor suppressor (hDlg) (Kiyono et al., 1997; Lee et al., 1997), scribble tumor suppressor (hScrib) (Nakagawa and Huibregtse, 2000) and Membrane-associated guanylate kinase inverted 1 (MAGI-1) (Dobrosotskaya et al., 1997; Ide et al., 1999). hScrib is involved in epithelial tight junctions and mediates the adhesion of basal cells to the extra-cellular matrix (ECM). Similarly, hDlg is involved in epithelial tight junctions, cell-to-cell junctions and epithelial polarity. While MAGI-1 has been suggested to co-localize with components of adherens junctions and tight junctions and its expression probably promotes the assembly of macromolecular junctional complexes. hDlg, hScrib and MAGI-1 are tumour suppressors; loss of these proteins facilitates cancer formation (reviewed in (Javier, 2008; Subbaiah et al., 2011)), and all high-risk E6 proteins target them for proteasome-mediated degradation (Gardioli et al., 1999; Glaunsinger et al., 2000; Nakagawa and Huibregtse, 2000), most likely leading to the loss of cell polarity and facilitating tumor formation (Pim et al., 2012; Thomas et al., 2008). In addition, recent studies have shown that high-risk E6 can interact with sorting nexin 27 (SNX 27), a component of the retromer complex and the endocytic transport machinery, again through its PBM. The interaction has been shown to impact upon the rates of cargo recycling and in maintaining high levels of glucose uptake in HPV-transformed cells (Ganti et al., 2016).

The E6 PBM is further regulated by post-translational modification by phosphorylation at S/T at the -2 position either by PKA, AKT or Chk1 kinases, resulting in inhibition of E6 PDZ binding activity and instead allowing E6 to associate with 14-3-3 proteins (Boon and Banks, 2013; Boon et al., 2015; Kuhne et al., 2000; Thatte et al., 2018). In HPV transformed cells, the steady state levels of phosphorylated E6 are low, however, under exposure to oxidative stress or

induction of DNA damage, there is a dramatic increase in the levels of phosphorylation of E6. This phosphorylation of E6 appears to involve the ATM/ATR signaling pathway resulting in E6 being phosphorylated directly by Chk1 or indirectly by Chk2 via PKA. Consequently, an intact phospho-acceptor site was shown to be important for E6's ability to inhibit p53 transcriptional activity on a subset of p53-responsive promoters, in a manner independent of E6's ability to direct p53 degradation (Thatte et al., 2018).

Modulation of cellular signaling pathways

The E6 protein also modulates several survival pathways, including phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT), Wnt and Notch. The PI3K/AKT pathway is a major cancer survival pathway regulating a broad range of downstream targets, including proliferation, cell growth, cell mobilization, angiogenesis and cell survival (Martini et al., 2014). E6 has been shown to inactivate PTEN, leading to increased pAKT and increased cell proliferation (Contreras-Paredes et al., 2009). The mammalian target of rapamycin (mTOR) kinase, downstream target of AKT, has been demonstrated to be activated by E6 via E6/E6AP-mediated degradation of mTOR inhibitor tuberous sclerosis complex 2 (TSC2) (Lu et al., 2004; Zheng et al., 2008). While under conditions of nutrient deprivation, HPV-16 E6 expression was shown to increase mTOR1 activity as a result of upstream activation of mTOR2 and 3-phosphoinositide-dependent kinase 1 (PDK1), leading to activation of AKT (Spangle and Munger, 2010). Furthermore, HPV-16 E6 expression was shown to sustain activation of receptor protein tyrosine kinases, including epidermal growth factor receptor (EGFR), insulin receptor beta and insulin-like growth factor receptor beta, mediated via the signaling adaptor protein Growth Factor Receptor-Bound protein 2 (GRB2), which are upstream of PI3K/AKT pathway (Spangle and Munger, 2013).

Further, nuclear accumulation of β -catenin has been shown to be associated with E6 in activation of the Wnt pathway. The mechanism was shown to be dependent on E6 ability to interact with E6AP and was shown to be independent of E6's ability to target p53 for degradation or to bind to the PDZ-containing E6 targets (Lichtig et al., 2010). Another mechanistic explanation of E6 activation of Wnt

pathways was shown to be through downregulation of seven in absentia homologue (Siah-1), involved in proteasomal degradation of β -catenin (Rampias et al., 2010). While, mice expressing wild-type E6 under the Keratin 14 promoter (K14E6 mice) showed enhanced nuclear accumulation of β -catenin and the accumulation of cellular β -catenin-responsive genes, mice expressing E6 lacking the PDZ binding domain (K14E6 Δ PDZ) mice did not, indicating that E6 activation of Wnt signaling is in part PBM-dependent (Bonilla-Delgado et al., 2012).

HPV-16 E6 has also been shown to activate the Notch pathway by interacting with NFX1-123 and increasing its expression, which, in turn, increases Notch-1 mRNA levels in E6-expressing cells (Vliet-Gregg et al., 2013). Modulation of Notch-1 by E6 was further shown to be mediated via presenilin-1 in mouse and human primary cell lines expressing HPV-16 E6 (Weijzen et al., 2003), while, expression of HPV-16 E6 has been shown to prevent early fate commitment of human keratinocytes towards differentiation and promoting cell proliferation at high cell densities, through a combined inactivation of p53 and Notch-1 (Kranjec et al., 2017). Furthermore, studies in cutaneous papillomavirus E6 oncoproteins have shown that E6 represses Notch signaling by association with MAML-1, a coactivator and effector of Notch-induced transcription, and thereby delays keratinocyte differentiation (Brimer et al., 2017; Brimer et al., 2012; Tan et al., 2012; White and Howley, 2013). It is intriguing to note that whilst E6 proteins from cutaneous HPV types targets MAML through an LXXLL motif, E6 proteins from mucosal HPV types targets E6AP through the same mechanism. This suggests that this interaction motif appeared early in the evolution of these viruses relative to their respective tropism in the mucosal or cutaneous epithelium, in order to favor cellular environment amenable for viral replication (Brimer et al., 2017; Tan et al., 2012; White and Howley, 2013; Zanier et al., 2013).

Telomerase activation

High-risk E6 can activate telomerase by a mechanism independent of p53 degradation and was shown to be important for immortalization (Klingelhutz et al., 1994; Klingelhutz et al., 1996). E6 was shown to interact with hTERT and increases telomerase activation by a posttranscriptional mechanism (Liu et al.,

2009). E6-mediated hTERT activity was shown to be the result of a combinatorial binding effect of Myc and Sp1 transcription factors responsible for hTERT expression (Oh et al., 2001). Furthermore, E6 was shown to cooperate with NFX1-123 to increase hTERT activity (Vliet-Gregg et al., 2013) and to downregulate hTERT repressors, such as NFX1-91 (Gewin et al., 2004), p300 (James et al., 2006) and Maz (Xu et al., 2013). The expression of hTERT and the telomerase activity were shown to be positively correlated with cervical cancer initiation and progression (Branca et al., 2006; Mutirangura et al., 1998). The advantage of activation of telomerase by HPV is not entirely clear, however, as telomeres are associated with a variety of proteins associated with DNA damage repair (DDR) pathway (Wallace and Galloway, 2014), a possibility could be modulation of DDR pathway for HPV genome replication.

The HPV 16 E7 is a 98 amino acid long, heterogenous protein based on its structural and dynamic properties (Phelps et al., 1992). The spliced E6*1 transcripts has been suggested to be the responsible for translation of E7 (Tang et al., 2006), however, recent evidences of circular RNA which encompasses E7 oncogene (circE7) has been demonstrated to have significant contribution to E7 protein levels and transforming properties despite being less abundant species (~1–3% of total E7 transcripts) (Zhao et al., 2019). The N-terminus of HPV16 E7 has sequence and function homology to a portion of CR1 and to the entire CR2 region of adenovirus (Ad) E1a and related sequences in the simian vacuolating virus 40 large tumor antigen (SV40 TAg) (Figge and Smith, 1988; Matlashewski et al., 1987; Phelps et al., 1988). The CR2 homology domain includes a LXCXE (X is any amino acid) motif, which is the interaction site for the retinoblastoma tumor suppressor (pRB) and related pocket proteins (Dyson et al., 1989; Munger et al., 1989b), however, optimum interaction requires the residues in the CR3 domain as well (Huang et al., 1993; Todorovic et al., 2012; Wu et al., 1993). Adjacent to this motif is a consensus phosphorylation site for casein kinase II (CKII) at serine 31 and 32 position in case of HPV 16 E7 (Barbosa et al., 1990; Firzlaff et al., 1989) (Figure 5).

HPV-16 E7

1 15 37 98

NH₂- CR1 CR2 CxxC CR3 CxxC -COOH

58 91

Ad5 Ea1 37 HFEP**PTLHE**-LYDL 49 116 VPEVID**LTCHE**AGFP**PSDD**EDE 137
 HPV-16 E7 2 HGDT**PTLHE**YMLDL 15 16 Q**PETTDLYC**YEQ**LNDS**SEEEDE 37
 SV40 TA_g 7 REESLQLMD-**LLGL** 19 98 FNEE-N**LFCSE**EM-**PSSDD**DET 117

CR1 CR2

(A). The position of conserved regions (CR1, CR2 and CR3) and CXXC (Cys-X-X-Cys) motifs, which participate coordinating zinc ion are also indicated. (B). Sequence alignment between CR1 and CR2 of HPV 16-E7, adenovirus type 5 E1a (Ad5E1a) and simian vacuolating virus 40 large

tumour antigen (SV40 Tag). Conserved residues are in bold letter and boxed region indicates the consensus LXCXE motif that targets pRB and pocket proteins p107 and p130.

Further to the C-terminus, the CR3 region contains two CXXC zinc-binding motifs separated by 29 amino acids (Barbosa et al., 1989; McIntyre et al., 1993). Unlike the N-terminus which is intrinsically disordered and is characterized by high flexibility (Calcada et al., 2013; Garcia-Alai et al., 2007; Ohlenschlager et al., 2006), the C-terminus appears to be more structured and is also responsible for formation of a homodimer as shown by the 3D structure of E7 CR3 regions from HPV-1A (Liu et al., 2006) and HPV-45 (Ohlenschlager et al., 2006). E7 has also been shown to form dimers (Clemens et al., 1995; McIntyre et al., 1993; Todorovic et al., 2011), tetramers (Clements et al., 2000) and higher order oligomers (Alonso et al., 2004; Alonso et al., 2006).

The HPV-16 E7 was shown to be a cytoplasmic phosphoprotein in 1987 (Smotkin and Wettstein, 1987), however, nuclear pools have also been reported (Greenfield et al., 1991; Guccione et al., 2002; Sato et al., 1989; Smith-McCune et al., 1999). Furthermore, recently E7 has been shown to be present in different subcellular (ER, Golgi and nucleus) compartments based on immunofluorescence techniques using antibodies recognizing different epitopes in HPV-16 E7 (Valdovinos-Torres et al., 2008). E7 proteins lack a typical nuclear localization sequence, however, it has been shown to gain entry to the nucleus through a RAN-dependent pathway (Angeline et al., 2003). While, RAN dependent nuclear export of E7 has been mapped to a well conserved residue (amino acids 76-84) in HPV E7 proteins (Knapp et al., 2009). Furthermore, E7 is post-transcriptionally regulated by the proteasome and by phosphorylation (discussed in section 'PTMs of HPV E7 and their role in HPV biology and carcinogenesis').

Role of HPV E7 in cell transformation

The cellular transforming activities of high-risk HPV genomes was established in mid 1980s in rodent cell transformations assays (Matlashewski et al., 1987; Yasumoto et al., 1986) and subsequently, E7 was recognized as the major transforming protein of high-risk HPVs using mutational analyses in such assays (Bedell et al., 1989; Kanda et al., 1988; Phelps et al., 1988; Tanaka et al., 1989;

Vousden et al., 1988; Watanabe and Yoshiike, 1988; Yutsudo et al., 1988). Later, using primary human keratinocytes, it was shown that high-risk HPV genomes causes life span extension, inhibit keratinocyte differentiation and leads to cellular immortalization (Schlegel et al., 1988; Woodworth et al., 1988; Woodworth et al., 1989). Organotypic raft cultures expressing the HPV genome was also shown to have cellular alterations and abnormalities in tissue architecture as in high-grade HPV-associated clinical lesions (McCance et al., 1988; Waggoner et al., 1990). While, the resulting cell lines were immortalized, they were not tumorigenic in nude mice but could induce tumors after several passages or in cooperation with additional oncogenes such as *ras* or *fos* (Bedell et al., 1989; Hawley-Nelson et al., 1989; Hudson et al., 1990; Munger et al., 1989a). Further mutational analysis showed that E7 protein in cooperation with E6 is necessary for these transforming activities in human keratinocytes, while such transforming and immortalizing activities are highly decreased in case of low-risk E7s (Barbosa et al., 1991; Halbert et al., 1992).

As with E6, E7 has not been reported to have any intrinsic enzymatic activity but is able to interact and subvert many different cellular regulatory proteins involved in cell cycle control and regulation, apoptosis, DNA damage and repair, transcription and many other vital cellular processes. Some of the key target proteins interacting with HPV-16 E7 are shown in a schematic diagram in Figure 6. Following sections below is aimed to discuss some of these key targets of E7 and their functions in cellular transformation.

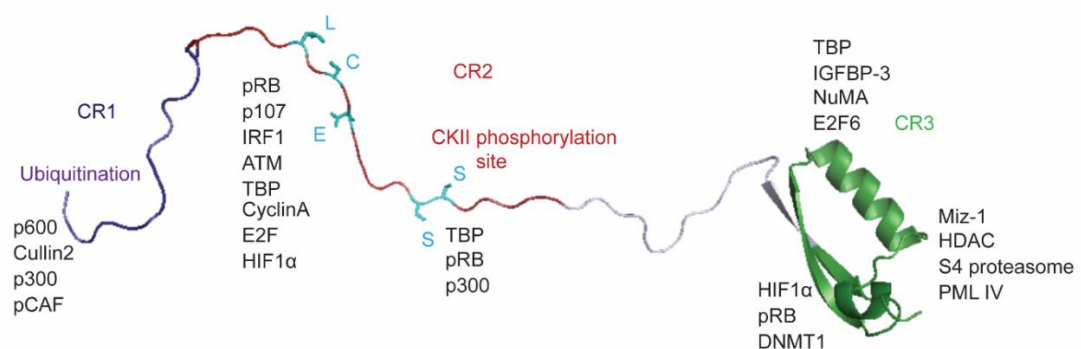


Figure 6. Hypothetical model of HPV-E7 showing various cellular binding partners.

Then N-terminus of E7 is intrinsically disordered and its structure has not been determined while the structure of C-terminus has been determined. Some of the key targets that E7 interacts are

p600 in the CR1 region shown to have role in anchorage independent growth and proteasomal degradation of PTPN14, pRB in the CR2 region (LXCXE motif) important for cell cycle entry, while regions in the CR3 region of E7 also contribute to pRB binding. (Refer to text for details).

Association of E7 with pRb and activation of E2F-dependent transcription

The members of the pocket family of proteins and their interactions with member of the E2F family of transcription factors plays a key role in regulation of cell cycle and apoptosis (Dyson, 1998). In normal cells, the association of pRB with E2F transcription factors blocks the transition of G1/S checkpoint of cell cycle until the cell receives a signal to divide (Frolov and Dyson, 2004). The G1 specific pRB/E2F transcriptional repressor complex is disrupted by phosphorylation of pRB by cdk4/6 and cdk2 in late G1 and the dissociated E2F acts as a transcriptional activator of genes necessary for S-phase entry and progression. In HPV infected cells, high-risk E7 can bind the G1 specific, E2F bound pRB and cause disruption of this repressor complex leading to uncontrolled G1 exit and S-phase entry (Dyson et al., 1992). pRB binds to the LXCXE motif in CR2 region of E7 while sequences in the CR3 regions of E7 have also been shown to be important (Huang et al., 1993; Wu et al., 1993). In addition, high-risk E7 are able to destabilize pRB (Boyer et al., 1996; Jones et al., 1997b) through proteasomal degradation by interacting with cullin 2 ubiquitin ligase complex (Huh et al., 2007) (Figure 7). Sequences in the CR1 region of E7 has also been shown to be necessary for pRB destabilization, in addition to LXCXE binding motif in the CR2 (Jones et al., 1997b).

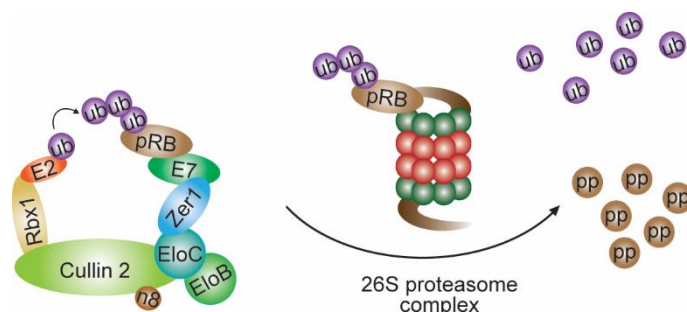


Figure 7. Hypothetical model of HPV-16 E7/Cullin 2 complex.

HPV-16 E7 associates with the Cullin 2 complex and recruits cellular proteins, including pRB (depicted in the model), p130 for ubiquitination and proteasome mediated degradation. Cullin 2-associated ubiquitin ligase activity may be increased in HPV-16 E7-expressing cells due to increased cullin 2 neddylation. ub – ubiquitin, aa – amino acids, n8 – nedd8 [adapted from (Huh et al., 2007)].

Furthermore, HPV-16 E7 can target multiple members of the E2F transcription factor family, including the transcriptional activator, E2F1 and the transcriptional repressor, E2F6 (Hwang et al., 2002). By interacting with E2F1, HPV-16 E7 can enhance E2F1-mediated transcription. E2F1 plays a role in mediating the transcriptional control of E2F6 gene, which is upregulated in the G1/S phase transition in order to repress E2F-responsive promoters, thereby checking the cell cycle for differentiation (Lyons et al., 2006). While, HPV-16 E7 associates with E2F6 and perturbs its ability to function as transcriptional repressor (McLaughlin-Drubin et al., 2008). Taken together, due to these functions of E7, the cells committed to exit cell cycle and differentiate are then able to remain in a S-phase-competent state necessary for viral life cycle. However, the resulting unscheduled DNA replication also activates the cellular apoptotic pathways by a mechanism termed ‘trophic sentinel response’ (Evan and Vousden, 2001) but is efficiently inactivated by high-risk E6 proteins (discussed in “E6 oncoprotein” section).

High-risk and low-risk E7s have differential ability in binding to members of the pRB family of proteins. Low risk E7 proteins bind to pRB with a much lower efficiency (approx. 10-fold less) than the high-risk HPV E7 proteins (Gage et al., 1990; Munger et al., 1989b). Again, only high-risk E7s can target all three pRB family members for degradation. Therefore, it has been suggested that the inability of low-risk HPV types to drive robust basal cell proliferation is because these types can only efficiently target p130 for degradation, which regulates cell cycle entry in the upper epithelial layers, but cannot target p107 and p105, which regulates the cell cycle in the basal and parabasal layer (Barrow-Laing et al., 2010; Boyer et al., 1996; Demers et al., 1994; Gonzalez et al., 2001; Helt and Galloway, 2001; Jones and Munger, 1997; Roman, 2006; Zhang et al., 2006).

Although E7’s oncogenic potential correlates more closely with the ability to induce pRB destabilization than just the interaction potential (Giarre et al., 2001;

Gonzalez et al., 2001), it has been shown that the destabilization of the pocket proteins is not sufficient to overcome cell cycle arrest or efficient cell transformation (Banks et al., 1990; Helt and Galloway, 2001; Shin et al., 2012). These observations highlight the importance of additional cellular targets of E7 involved in stimulating cell cycle progression and suggests multiple mechanisms are involved in inducing and maintaining of cell transformation.

G1/S cell checkpoint deregulation by other mechanisms

In addition to destabilization of pRB leading to perturbation of pRB/E2F complex, E7 further enhances G1/S transition by binding with both positive and negative regulators of the cell cycle. Cyclin dependent kinases (cdks) are drivers of the cell cycle. The regulatory subunits of cdk2, cyclin E and A, which drive S-phase entry and progression, is under the control of E2F. And it has been shown that cells expressing E7, maintain high levels of both cyclin E and A as a result of destabilization of pRB and increased E2F dependent transcription (Zerfass et al., 1995). E7 can also directly associate with cdk2/cyclin A and cyclin E complexes resulting in increased cdk2 activity (He et al., 2003; Nguyen and Munger, 2008; Tommasino et al., 1993). Furthermore, HPV16 E7 also causes an increase in the transcription of the cell division cycle (cdc) 25A phosphatase involved in removing inhibitory phosphorylation of cyclin E and A complexes, leading to further activation of cdk2 activity (Katich et al., 2001). More recently, cdc6 was also shown to be up-regulated in E7-expressing cells (Fan et al., 2016), which promotes cell cycle progression by activating cdk2 (Kan et al., 2008; Uranbileg et al., 2012) and further cdc6 was shown to be important in G1/S transition in E7-expressing cells in hypoxic conditions (Chen et al., 2017).

During keratinocyte differentiation, loss of contact with the basal membrane is accompanied by increased levels of cyclin dependent kinase inhibitors (CKIs), subsequently inhibiting cdk2 activity inducing a G1 growth arrest. However, HPV 16 E7 abrogates the inhibition of cdk2 activity by interacting with CKIs, p21^{Cip1} (Funk et al., 1997; Jones et al., 1997a) and p27^{Kip1} (Zerfass-Thome et al., 1996), which are induced by anti-proliferative signals, including growth factor withdrawal (Firpo et al., 1994), activation of p53 (el-Deiry et al., 1993) and loss of cellular adhesion (Assoian, 1997; Fang et al., 1996). Although E7 expression increases

p21^{Cip1} through protein stabilization (Jian et al., 1998; Jones et al., 1999; Noya et al., 2001), cdk2 remains active in HPV-E7 expressing cells (Funk et al., 1997; Jones et al., 1997a; Ruesch and Laimins, 1997). Furthermore, abrogation of p21^{Cip1} inhibition has been shown to require C-terminus sequences of E7, where zinc binding site mutants are shown to be proficient for targeting pocket pockets for degradation but are yet unable to overcome growth arrest (Helt et al., 2002; Helt and Galloway, 2001). Further, this function of abrogation of p21^{Cip1} activity is less efficient in low-risk E7 (Funk et al., 1997; Jones et al., 1997a). Thus, the ability of HPV E7 to abrogate CKIs and disrupt pRB/E2F complexes resulting in increased levels of cyclin A and E, together establishes a replication competent cellular milieu in differentiating keratinocytes necessary for virus propagation.

HPV E7, senescence and autophagy

Repression of endogenous E6 and E7 using bovine papillomavirus E2 protein and re-expression of high-risk HPV E7 protein in HeLa cells, results cells in proliferation, senescence or apoptosis depending on the particular mutant of E7 analysed (DeFilippis et al., 2003). Mutational analysis of high-risk E7 in the CR1 region (16E7 Δ 6-10) and CR2 region (16E7 Δ 21-24) indicated that Rb binding was not enough to prevent senescence induced by repression of endogenous E7 in HeLa cells but degradation of RB was also required (P syrri et al., 2004). High-risk E7 has also been shown to upregulate DEK proto-oncogene, a negative mediator of senescence (Wise-Draper et al., 2005). While, promyelocytic leukemia protein IV (PML IV), a positive mediator of senescence, has been shown to be inhibited by both high-risk and low-risk E7. This activity was shown to be dependent on Rb binding site and also CR3 regions of E7 (Bischof et al., 2005).

Furthermore, high-risk HPV E7 has been shown to induce autophagy in response to growth factor deprivation (Zhou and Munger, 2009), which might be a result of metabolic stress due to sustained proliferative activity and/or reprogramming of the cellular metabolism to a less efficient anaerobic mechanism (Zwerschke et al., 1999). This switch to a lesser requirement of oxygen and increasing glycolysis has been linked to interaction of high-risk E7 C-terminus to M2

pyruvate kinase resulting in the less active form of the enzyme (Zwerschke et al., 1999). Thus, induction of autophagy may be a cellular response to enhanced proliferation in limited growth factors conditions or E7-induced increased requirement for energy (Zhou and Munger, 2009; Zhou et al., 2009).

E7 and genomic instability

Despite the fact that expression of high-risk oncogenes in primary human keratinocytes causes cellular immortalization exhibiting many characteristics of premalignant lesions, these cells do not form tumors when injected into nude mice. Additional oncogenic events are necessary for malignant progression to occur, such as expression of oncogenes like *ras* or *fos*, or accumulation of oncogenic mutations over prolonged passaging in culture (DiPaolo et al., 1989; Durst et al., 1989; Hurlin et al., 1991; Pei et al., 1993).

High-risk HPV E7 has been suggested to cause genomic instability (Duensing et al., 2000). HPV-16 E7 has been shown to induce centrosomal duplication errors leading to multipolar mitoses, chromosome mis-segregation and aneuploidy, independently of RB-inactivating activity (Duensing and Munger, 2003). Further, HPV-16 E7 was shown to associate with the centrosomal regulator, γ -tubulin, altering the recruitment of gamma-tubulin to the centrosome in HPV-16 E7 expressing cells, suggesting a role of E7 in abnormal centrosomal amplification and disrupting centrosome homeostasis (Nguyen et al., 2007). Abnormal centriole multiplication was also shown to correlate with up-regulation of Polo-like kinase 4 in HPV-16 E7 expressing cells (Korzeniewski et al., 2011). In addition, HPV-16 E7 expression was shown to induce delocalization of dynein from mitotic spindles (Nguyen et al., 2008) and induce aneuploidy in infected cells by disrupting nuclear mitotic apparatus protein 1 (NuMA)/dynein network even in the absence of supernumerary centrosomes (Nguyen and Munger, 2009).

Further, high-risk E7 has been shown to target ATM/ATR DNA damage response pathways. HPV-31 E7 was shown to bind ATM inducing its phosphorylation and activating Chk2 (Moody and Laimins, 2009). HPV-18 E7 was shown to induce increased levels of phosphorylated ATM and the downstream kinases Chk1, Chk2 and JNKs (c-jun N-terminal kinases) (Banerjee et al., 2011). In addition,

high-risk E7 was shown to target claspin, a key regulator of ATR-Chk1 pathway activated in response to replication stress. HPV-16 E7 was shown to attenuate mitotic checkpoint control by upregulating cellular factors involved in destabilization of claspin – a positive regulator of mitotic checkpoint, primarily dependent on the ability of E7 to inactivate RB, as most of the factors involved in turnover of claspin are regulated by E2F transcription factors (Spardy et al., 2009). Thus, E7 induced accelerated degradation of claspin in G2/M phase leads cells to initiate checkpoint recovery even in presence of DNA damage potentially leading to genomic instability (Spardy et al., 2009) and contributing to E7 mediated carcinogenesis.

Furthermore, HPV oncogenes hinder the homologous-recombination repair pathway, where HPV E7 impairs RAD51 localization to double strand break (DSB), leading to inability to robustly repair DSBs, causing some of these lesions to be more persistent and contributing to genomic instability (Wallace et al., 2017). More recently, HPV E7 has been shown to directly interact with E3 ubiquitin ligase RNF168, a critical factor in DNA repair following DSBs. This interaction leads to perturbation of cellular DSB signaling where, HPV E7 hijacks this ligase to promote viral replication cycle (Sitz et al., 2019).

HPV E7 and Epigenetic reprogramming

Alterations in DNA methylation are associated with human diseases and are one of the hallmarks of cancer. The HPV-16 E7 oncoprotein has been shown to directly bind to DNMT1 and stimulate its methyltransferase activity (Burgers et al., 2007). Further, HPV E7 proteins have been shown to interact with both HATs and HDACs. HPV-16 E7 has been shown to interact with p300/CBP-associated factor (PCAF) histone acetyltransferase (Avvakumov et al., 2003) and this activity has been shown to contribute to downregulation of IL-8, which might contribute to the ability of infected cells to avoid the host immune response (Huang and McCance, 2002).

HPV E7 has also been shown to interact with HDAC1 and HDAC2 through Mi2 β protein (Brehm et al., 1999). Both Mi2 β and HDAC1/HDAC2 are components of the NuRD chromatin remodeling complex. This interaction has been demonstrated to modulate histone modification and transcription of cellular

genes relevant to cell cycle deregulation (Nguyen et al., 2002) or immune evasion (Park et al., 2000). HPV-16 E7 interacts with interferon regulator factor-1 (IRF-1), which activates IFN- β gene, however, by recruiting HDAC to abrogate transactivation function of IRF-1, E7 has been suggested to suppress a cellular immune response to HPV infection (Park et al., 2000). Further, the association of HPV-31 E7 and HDACs in differentiated cells is involved in activation of E2F2 gene transcription facilitating HPV31 replication (Longworth et al., 2005). HPV E7 has also been shown to enhance HIF-1 α -dependent transcription by inducing dissociation of HDAC1, HDAC4 and HDAC7 from HIF-1 α , which might contribute to tumour angiogenesis (Bodily et al., 2011b).

HPV E7 has also been shown to induce expression of histone H3 lysine 27 demethylase, KDM6A and KDM6B enzymes responsible for H3K27me3 demethylation (McLaughlin-Drubin et al., 2011). Further, KDM6B induction mediates increased expression of cervical cancer biomarker p16^{INK4A}. Higher expression of p16^{INK4A} caused by HPV-16 E7 mediated by KDM6D upregulation represents an E7-triggered oncogene-induced senescence (OIS) response. This response, as RAS/RAF cause KDM6B upregulation, leads to de-repression of p16^{INK4A} transcription followed by inhibition of CDK4/6 activity and inhibition of pRB phosphorylation. The ultimate effect is G1 cell cycle arrest and senescence, however, since, HPV E7 targets pRB for ubiquitin-dependent proteasomal degradation and this explains why p16^{INK4A} upregulation in HPV positive cancer cells does not inhibit proliferation (McLaughlin-Drubin and Munger, 2013; McLaughlin-Drubin et al., 2013; Munger et al., 2013). Further, KDM6A- and KDM6B-responsive Homeobox (HOX) genes are expressed at significantly higher levels, suggesting that ectopic expression of HPV-16 E7 results in reprogramming of host epithelial cells (McLaughlin-Drubin et al., 2011). Furthermore, increased KDM6A in response to high-risk HPV E7 expression was shown to cause de-repression of cell cycle and DNA replication inhibitor p21^{CIP1} and this activity was shown to be required for high-risk E7 expressing cells for p21^{CIP1}'s ability to inhibit DNA replication through PCNA binding (Soto et al., 2017).

The polycomb group of proteins forms polycomb repressive complexes (PRC) and repress gene transcription (Bracken and Helin, 2009). PRC2, for instance,

silences genes by trimethylating lysine residue 27 of histone H3, while PRC1 binds to H3K27me₃-marked chromatin and further silences gene expression by monoubiquitinating lysine K119 of histone H2A. HPV-16 E7 associates with E2F6 factor with multiple polycomb group of proteins including BMI1, PCGF2 (MEL-18), CBX4 (hPC2), RING1, MGA, and L3MBTL2, and abrogates repressive activity of E2F6 on its target genes (McLaughlin-Drubin et al., 2008). HPV-16 E7 has also been shown to induce expression of H3K27 histone methyltransferase EZH2 (enzymatic component of PRC2), enhancing PRC4 complex formation (Holland et al., 2008), which has been demonstrated to cause histone H1K26 deacetylation and methylation (Kuzmichev et al., 2005).

Modulation of micro RNAs by HPV E7

Among many other factors, micro RNAs (miRNAs) are also known to regulate the expression and activities of cellular proteins by acting as post-transcriptional regulators of gene expression (Suzuki et al., 2012). These are small RNA molecules (18-25 nucleotides), transcribed by RNA polymerase II. HPV E7 has been shown to downregulate miRNA203, which is normally expressed in higher levels in differentiating cells to downregulate the p63 family of transcription factors and inhibit cell proliferation. However, E7 mediated downregulation of miRNA203 appears to be necessary for genome amplification and productive replication in differentiating cells (Melar-New and Laimins, 2010). In addition, a number of other micro RNAs are modulated by expression of E6 and E7 oncoproteins, singly or in combination. Specifically, upregulation of miR-16-2-3p and downregulation of miR-197-3p and -1249 in HPV-16 E6/E7 expressing HFKs is driven by E7 expression (Harden et al., 2017). These microRNAs together with others being modulated by HPV oncoproteins seem to affect several of the cellular signaling pathways including p38 MAPK signaling, G1/S checkpoint regulation and ATM signaling, thus contributing towards rewiring of cellular regulatory pathways in oncogenic transformation (Harden et al., 2017; Yablonska et al., 2013).

UBR4/p600, Anoikis and anchorage independence

High-risk and low-risk HPV E7, as well as E7 protein from bovine papillomavirus 1 (BPV1) has been shown to associate with p600/UBR4 (DeMasi et al., 2005;

Huh et al., 2005). p600 has been implicated in regulation of anoikis signaling. Anoikis is a form of apoptosis mediated in normal cells when they attempt to divide in absence of a matrix (Frisch and Screaton, 2001). Interaction of E7 with p600 has been shown to be through CR1 amino-terminal domain of E7 (DeMasi et al., 2005; Huh et al., 2005), which is necessary for the transforming ability of HPV-16 E7 (Gulliver et al., 1997; Phelps et al., 1992) and expression of the BPV-1 E7 oncoprotein has been suggested to result in resistance to anoikis (DeMasi et al., 2007). Further, p600 is a E3 ubiquitin ligase and is involved in the N-end rule pathway in ubiquitin proteasome pathway (Tasaki et al., 2005). More recently p600/UBR4 interaction with high-risk E7 was shown to be important for destabilization of PTPN14 (Szalmas et al., 2017; White et al., 2016).

PTMs of HPV E7 and their role in HPV biology and carcinogenesis

Most nascent proteins undergo several modifications before they can fulfil their various functions as enzymes, or as structural biomolecules involved in maintaining diverse cellular functions. Many others undergo a series of sequential modifications to relay signals from the extra-cellular milieu to the cell interior, leading to alterations in gene expression and function. Phosphorylation, ubiquitination, glycosylation, acetylation, proteolysis, deamidation are a few of the many post-translational modifications (PTM) that have been extensively studied. Given a complex network of continuous signals being relayed in the cell, most proteins are continually undergoing one or several of these modifications, and this also applies to several other proteins that are exogenously acquired – for example, proteins from viral gene expression. In case of HPV, PTMs of viral proteins play a significant role in the viral life cycle and in carcinogenesis, as noted above in the case of E6. The following sections are aimed to summarize the reported PTMs of the HPV E7 oncoprotein in terms of the viral life cycle and cell transformation.

As discussed previously, many functions of the E7 protein are mediated through interaction with various cellular proteins, and some of these interactions are strongly modulated by post-translational modifications of E7. Despite much previous research being mainly focused on HPV biology and HPV-induced carcinogenesis, the specific role of PTM of viral proteins and oncogenes in these processes has not been well explored.

Phosphorylation

HPV 16E7 has long been recognized as a cytoplasmic/nuclear serine phosphoprotein (Smotkin and Wettstein, 1987). HPV E7 contains a consensus phosphorylation site for CKII (Figure 8) at the N-terminus of the protein in the CR2 homology domain (Barbosa et al., 1990; Firzlaff et al., 1989). The substrate specificity for CKII recognition is determined by multiple acidic residues located between the -2 and +5 positions, relative to the S/T phospho-acceptor site (Marin et al., 1994; Sarno et al., 1996). Although a run of three acidic amino acids is enough for CKII recognition, a stretch of five acidic downstream residues, as is found in HPV-16 E7, has been found to be more efficient (Marin et al., 1986).

Both the serine residues of HPV-16 E7 at S31 and S32 have been demonstrated to be phosphorylated by CKII (Figure 8) (Barbosa et al., 1990; Firzlaff et al., 1989). In addition, recently, a variant of 16E7 with an additional serine at S29 has also been shown to be phosphorylated by CKII (Zine El Abidine et al., 2017). Similar consensus phosphorylation sites in HPV-18, HPV-11 and HPV-6 E7 proteins have been shown to be phosphorylated (Armstrong and Roman, 1995; Firzlaff et al., 1989; Genovese et al., 2008); however, CKII phosphorylation kinetics were shown to be twice as fast for HPV-18 E7 as for HPV-16 E7, which, in turn, was twice as fast as for HPV-6 E7 (Barbosa et al., 1990).

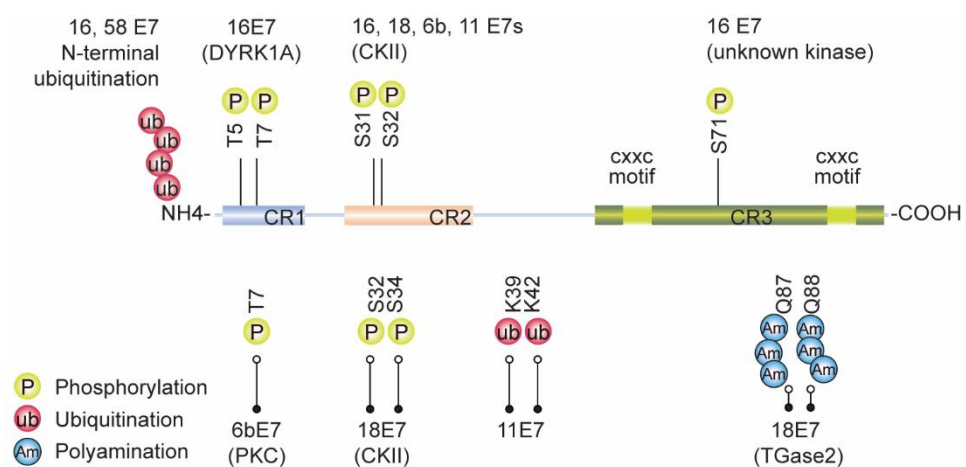


Figure 8. Schematic representation of the known HPV E7 post translational modifications.

HPV E7 can be phosphorylated, ubiquitinated and polyaminated (refer to text for details). CR – conserved region, CKII – casein kinase II, DYRK1A – dual-specificity tyrosine phosphorylation-regulated kinase 1A, PKC – protein kinase C, p – phosphate group, ub – ubiquitin, Am – amine group.

The 3D structures of the C-terminal halves of E7 proteins from HPV-1A (Liu et al., 2006) and HPV-45 (Ohlenschlager et al., 2006) have been determined, while the N-terminal halves have been termed heterogenous in structural behavior and characterized as intrinsically disordered and with high flexibility (Calcada et al., 2013; Ohlenschlager et al., 2006). However, recent structural studies have revealed that CKII phosphorylation of E7 at these residues does not affect E7's overall conformation, but is most likely to contribute to local changes in charges at serine residues S31 and S32, thus affecting E7's capacity to interact with its target proteins (Nogueira et al., 2017). Indeed the N-terminal half of HPV E7(1-40) displays an extended polyproline II (PPII) conformation, which is not only fully

accessible to PTMs, but is also stabilized by CKII phosphorylation at S31/S32, further increasing the PPII content and promoting the accessibility of this interaction motif for binding with various cellular interacting partners (Chemes et al., 2010; Garcia-Alai et al., 2007; Kukic et al., 2019).

In line with the above-mentioned details, the clue to phosphorylation of E7 and enhanced binding initially came from structural studies of pRB and the E7 binding region (Lee et al., 1998) (Figure 9). The LXCXE motif upstream of the CKII phospho-acceptor site in E7's CR2, binds pRB and is robustly engaged via the additional negative charges of the CKII-phosphorylated S31/S32 residues of E7 (Chemes et al., 2010; Dick and Dyson, 2002; Dyson et al., 1989; Lee et al., 1998; Singh et al., 2005). Furthermore, an intact CKII phosphorylation site has been shown to be important for E7 to be able to target and inactivate pRB and related pocket proteins by proteasomal degradation (Genovese et al., 2008; Jones et al., 1997b).

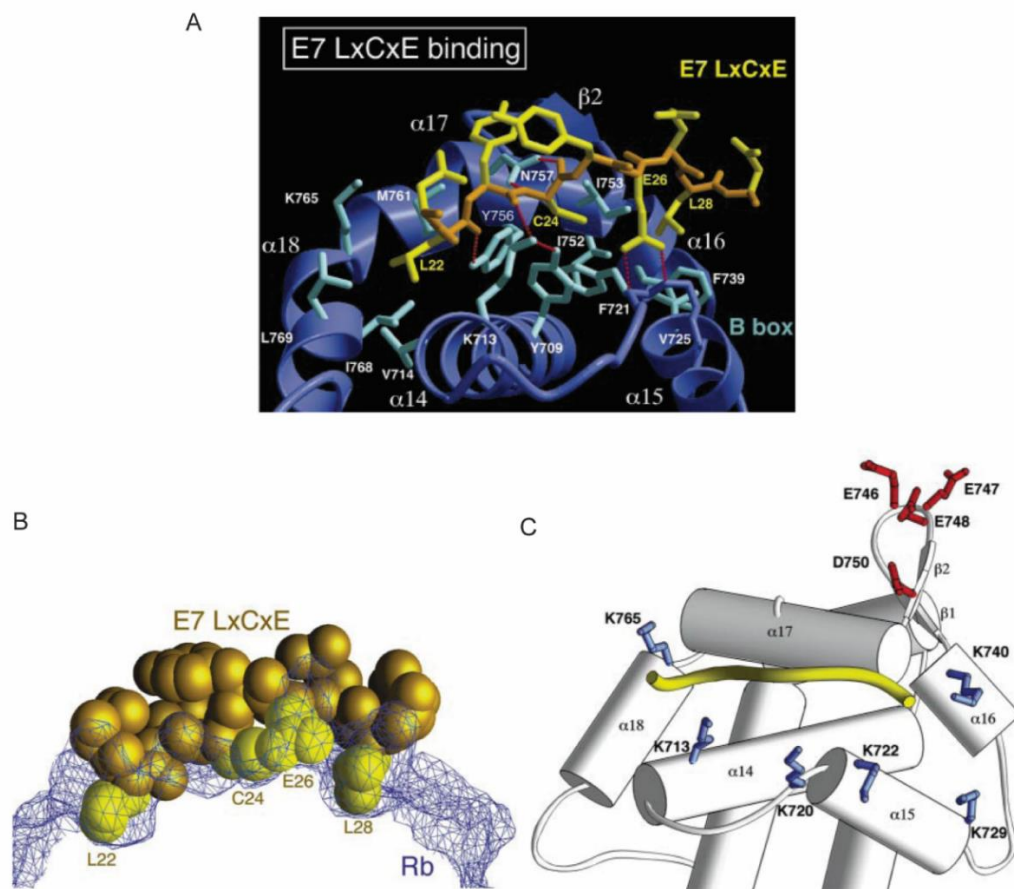


Figure 9. The E7 LXCXE binds as an extended peptide onto a conserved groove of B-box of RB (Lee et al., 1998).

A. Three-dimensional structure showing hydrogen-bonds interactions to peptide backbone groups (indicated as red dotted lines) of E7 LXCXE region with B-box of pRB. B. The E7 L22, C24 and L28 side chains bind in small hydrophobic pockets of the B-box of pRB. C. The clustering of basic residues at the rim of the LXCXE binding site, a well-conserved feature, facilitating binding of viral oncogenes that often have a stretch of acidic residues following the LXCXE motif. A conserved clustering of acidic residues at the opposite side of the binding site can also be observed.

A similar enhancement of E7's interaction with TATA Box Binding Protein (TBP) was also shown to be dependent on phosphorylation of HPV 16E7 by CKII, where a mutation to acidic residues instead of serine at the CKII phospho-acceptor site significantly increased the affinity for TBP (Massimi et al., 1996; Phillips and Vousden, 1997). It has been further suggested that E7 phosphorylation may play a role in E7 interaction with p53 in a complex with TBP that inhibits p53 transcriptional activity (Massimi and Banks, 1997). Moreover, HPV 16E7 was also shown to interact with F-actin in a CKII phosphorylation-dependent manner, with consequent decrease in polymerized actin and disruption of the actin cytoskeleton in cells expressing E7 (Rey et al., 2000).

CKII is an ubiquitous serine/threonine protein kinase that has been reported to localize in both cytoplasm and nucleus and has been shown to be associated with the plasma membrane, Golgi and endoplasmic reticulum (reviewed in (Litchfield, 2003)). CKII activity is modulated by growth factors (Ackerman and Osheroff, 1989) and can be inhibited by calprotectin (Murao et al., 1989). Calprotectin, or the S100 calcium-binding protein complex, is composed of macrophage-inhibitory related factor (MRP) proteins 8 and 14 and has been shown to inhibit CKII (Murao et al., 1989) and decrease CKII-dependent HPV 16E7 phosphorylation (Tugizov et al., 2005). Furthermore, MRP 8/14 levels were undetectable in HPV-immortalized human keratinocytes that had a four-fold increase in CKII activity compared with normal keratinocytes (Tugizov et al., 2005), suggesting a possible role for the complex in regulating CKII-mediated E7 phosphorylation.

Biologically, phosphorylation of HPV 16E7 S31/S32 residues by CKII, or their mutation to negatively-charged amino acids such as aspartic acid, has been shown to be important for cell transformation (Barbosa et al., 1990; Firzlaff et al., 1991; Heck et al., 1992); while in context of the whole HPV-16 genome, mutation

of the CKII phospho-acceptor site results in reduced virus production in raft cultures (Bodily et al., 2011a). In terms of cell proliferation and terminal differentiation, mutation of the CKII phospho-acceptor site to phosphorylation-deficient A31/A32 was shown to impair the ability of HPV-16 E7 to override the b-zip transcription factor CCAAT/enhancer-binding protein alpha (C/EBPalpha)-mediated cell cycle arrest, without affecting the transactivation activity of C/EBPalpha or its ability to participate in differentiation (Muller et al., 1999). In postmitotic and differentiated cells, negative charges or phosphorylation at one or both of the serines in CKII phospho-acceptor sites of E7 have been shown to be required for efficient activation of S-phase genes and subsequent induction of S-phase reentry (Chien et al., 2000; Genovese et al., 2008). All these functions described for the E7 CKII phospho-acceptor site relate to viral life cycle or to early events of transformation. In the case of cervical cancer derived cells, one study addressed the role of CKII phosphorylation in the maintenance of cell proliferation in HeLa cells. Using overexpression of E2 to suppress viral oncoprotein expression and subsequent rescue with E7 overexpression, an intact CKII phospho-acceptor site was found to be important for optimal cell growth (P syrri et al., 2004).

As with the E6 oncoprotein, phosphorylation of E7 does not appear to be constitutive and is differentially regulated during the cell cycle. CKII phosphorylation of E7 mainly occurs during the G1 phase at S31/S32 and decreases rapidly as the cells progress towards S-phase (Massimi and Banks, 2000). The same study also found evidence of an additional phospho-acceptor site in E7 at S71 in the carboxy-terminal half of the protein. In this case phosphorylation at S71 seemed to occur preferentially during S-phase, although the kinase responsible has not yet been identified (Massimi and Banks, 2000). Furthermore, the biological relevance of phosphorylation at S71 also remains to be clarified, although mutations at this site have been linked to reduction in induction of tetrasomy in raft cultures, when compared with wildtype E7-expressing cells (Southern et al., 2004). However, a clear link to tetrasomy with phosphorylation at S71 has so far not been demonstrated. Phylogenetically, this residue is highly conserved in high-risk alpha papillomaviruses, further

suggesting a conserved function for this residue that warrants further investigation.

HPV-16 E7 has also been shown to be phosphorylated *in vitro* and *in vivo* at threonine residues T5 and T7 in the CR1 homology domain, by dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), even when a perfect consensus recognition sequence [RXX(S/T)P] is not present at the phosphorylation site (Liang et al., 2008). Furthermore, this kinase was shown to bind to the C-terminus of HPV-16 E7 and increase E7 stability, resulting in an increased ability to target pRB for degradation. Phosphorylation by DYRK1A at T5 and T7 and subsequent increase stability of E7 has been suggested to be the result of the perturbation of N-terminal ubiquitination of E7 (see section "Ubiquitination of E7") (Liang et al., 2008).

In low-risk HPV-6 E7, the threonine residue T7 in the CR1 homology domain has been shown to be phosphorylated by protein kinase C (PKC), while the consensus threonine residue of HPV-16 E7 was not phosphorylated by PKC under similar *in vitro* conditions. Although this particular threonine residue at T7 seems to be well-conserved in many HPV types, including the high-risk E7s, the discrepancy in phosphorylation seems to reside in deviation of the PKC recognition sequence [(R/K₁₋₃/X₂₋₀)-S/T-(X₂₋₀/R/K₁₋₃)] in the high-risk types, suggesting a qualitative biochemical difference between high-risk and low-risk E7 (Armstrong and Roman, 1995).

Ubiquitination of E7

HPV E7 is a short-lived protein: turned over very quickly in cells (Selvey et al., 1994) via the ubiquitin proteasome pathway (Reinstein et al., 2000; Wang et al., 2001), and has a half-life of less than an hour (Reinstein et al., 2000; Wang et al., 2001). HPV-16 E7 has two internal lysine residues; however, mutating both lysine residues to arginine did not affect its ubiquitination or degradation. Furthermore, addition of a Myc tag to the N-terminal but not to the C-terminal residue was shown to stabilize the protein, suggesting that E7 is subject to N-terminal ubiquitination and proteasomal degradation (Figure 8) (Reinstein et al., 2000). The N-terminal ubiquitination of E7s is further supported by the case of HPV58 E7, which does not contain any lysine residue, but has been shown to be

ubiquitinated and degraded by the proteasome (Ben-Saadon et al., 2004). It has further been shown that the first 11 residues in the N-terminus of HPV 16E7 are important for its ubiquitination, and that deletion or N-terminal tagging stabilizes the protein (Reinstein et al., 2000). A functional interaction of E7 with the S4 subunit of the 26S proteasome has also been described, but whether this interaction has any role in E7 proteolysis is not clear (Berezutskaya and Bagchi, 1997; Wang et al., 2001). Whether ubiquitination of E7 plays any role in E7 function other than targeting it for degradation remains to be determined, however it is intriguing that N-terminally tagged E7 proteins are defective in transforming activity, despite their increased stability. Whether this is due to loss of ubiquitination or loss of ability to interact with other cellular substrates such as p600 (see below), remains to be determined.

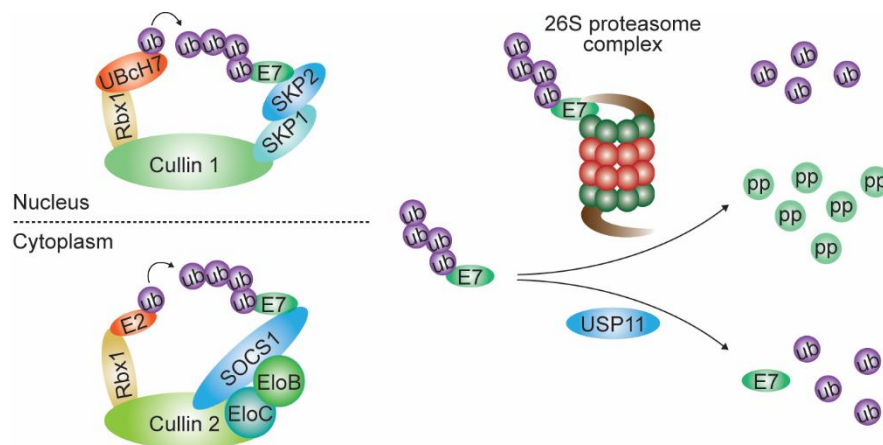


Figure 10. Post translational modification of HPV E7 by ubiquitin proteasome pathway.

Hypothetical schematic representation of the SOCS1 complex (in cytoplasm) and SCF E3 ligase complex (in nucleus) in ubiquitination and proteasomal degradation of E7 and stabilization of E7 by USP11 (refer to text for details). ub – ubiquitin, pp – 2-25 residue peptides.

E7 has also been shown to interact with suppressor of cytokine signaling-1 (SOCS1)/JAB, a member of the CIS/SOCS family of proteins, resulting in the destabilization of E7 by its ubiquitination in a SOCS-box-dependent manner (Figure 10) (Kamio et al., 2004). SOCS1 is a component of the VCB-type E3 ubiquitin ligase, containing Elongin B and C, which recruits Cullin-2, Rbx-1 and E2 ubiquitin transferase complex, and acts as an adaptor protein to recruit target proteins for ubiquitination and proteasomal degradation (Zhang et al., 1999). Thus, higher levels of E7 protein and consequent higher sensitivity to E7-

mediated transformation has been observed in SOCS1-deficient fibroblasts. However, E7 is still weakly ubiquitinated in the presence of a SOCS-box-defective mutant of SOCS1, suggesting possible involvement of other ubiquitin ligases (Kamio et al., 2004). Further, the ring E3 ligase, SCF (Skp-Cullin-Fbox) ubiquitin ligase complex, containing Cullin 1 and Skp2, was shown to associate with and destabilize E7 by ubiquitination and proteasomal degradation (Oh et al., 2004). In the same analysis, it was shown that E7 proteolysis was not completely blocked in Skp2-deficient cells, suggesting the involvement of Skp2-independent proteolysis of E7 (Oh et al., 2004). Several proteins, such as p27Kip1 and E2F-1, often require specific phosphorylation prior to SCF-dependent ubiquitination (Montagnoli et al., 1999; Ohta and Xiong, 2001), however, it is not clear if E7 requires phosphorylation prior to SCF-dependent ubiquitination (Oh et al., 2004). Even though two independent ubiquitin ligase pathways have been described, SOCS1-dependent destabilization of E7 occurs predominantly in the cytoplasm (Kamio et al., 2004), while SCF-dependent ubiquitination is involved in destabilization of nuclear pools of E7 (Oh et al., 2004), suggesting the involvement of complementary mechanisms of E7 destabilization. Furthermore, ubiquitination of HPV 16E7 is opposed by USP11 (ubiquitin specific protease 11). The C-terminus of USP11 binds E7 and increases its steady state levels by reducing ubiquitination of E7 and subsequent degradation (Lin et al., 2008).

Low risk HPV-6b E7 and HPV-11 E7 proteins have also been shown to be ubiquitinated, however, they have a longer turn-over time compared with high-risk E7s, based on cycloheximide pulse-chase experiments of estrogen-receptor fused HPV E7s. While HPV-11 E7 could be ubiquitinated at non-conserved lysine residues at positions 39 and 42, surprisingly, mutations to arginine residues rather decreased protein stability (Genovese et al., 2011).

Other modifications

Polyamination of HPV 18E7

HPV-18 E7, but not HPV-16 E7, has been shown to be modified by Transglutaminase 2 (TGase 2), which attaches polyamines onto the glutamine residues in the C-terminal region of E7 (Figure 8). While both HPV-16 and HPV-18 E7 have been shown to associate with TGase2, only HPV-18 E7 can undergo

polyamination, as there are no glutamine residues at those potential polyamination sites in HPV-16 E7. Interestingly, polyamination of the HPV-18 E7 protein by TGase 2 diminishes E7's ability to bind Rb, suggesting that this could be one possible explanation for the higher prevalence of HPV-16 than HPV-18 in cervical cancer (Jeon et al., 2006; Jeon et al., 2003). TGase2 belongs to a family of calcium-dependent enzymes that catalyze PTMs of proteins through a transamination reaction involving polymerization of polyamines onto proteins and/or deamidation of proteins (Lorand and Graham, 2003). Based on this finding, a study conducted to investigate modulation of TGase2 in infected human cervical epithelium showed that TGase2 was absent in normal squamous mucosa, but was present in CIN I lesions. Furthermore, TGase2 expression was higher in CIN I than in CIN II/III, suggesting that TGase2 expression in HPV infection is an early phenomenon and probably a part of the host cell reaction against HPV-induced tissue modification (Bazzo Goulart et al., 2017; Del Nonno et al., 2011).

The existence of additional PTMs of HPV E7 is demonstrated by the existence of multiple isoforms of E7 with different molecular weights and isoelectric points (IEP), described as E7a1 (17.5 kDa, IEP 4.68), E7a (17 kDa, IEP 6.18) and E7b (16 kDa, IEP 6.96). These isoforms have been shown to be distributed into the ER, Golgi and nucleus, suggesting the possibility of other posttranslational modifications of E7 besides phosphorylation (Valdovinos-Torres et al., 2008).

Therapeutic implications of studying E7 PTMs

Despite the availability of three potent prophylactic vaccines covering the majority of cancer-causing HPV types, there is still need of a therapeutic target against cervical cancer. This is because the current vaccines are prophylactic, not therapeutic, and thus would not be useful to an already-infected population who could potentially develop cancer over several years. In addition, there is a huge challenge in terms of both logistics and cost of the vaccine in many developing countries where most of the cases occur. Thus, a potential therapeutic target is urgently needed for HPV-induced carcinogenesis. The following section attempts to summarize recent therapeutic interventions against

HPV-induced cancers, targeting PTMs of E7 and/or their functions as one such strategy.

CKII and CKII inhibitors in HPV-associated cancers

CKII is a constitutively active, pleiotropic and highly conserved serine/threonine kinase (Allende and Allende, 1995; Pinna, 2002). The mammalian family of these kinases is composed of 2 genes *CSNK2A1* (CKII α) and *CSNK2A2* (CKII α'). CKII kinases can act monomerically or as a tetrameric complex composed of two CKII units (CKII α and/or CKII α') and two regulatory units (CKII β). The regulator subunit is encoded by a separate gene *CSNK2B* and regulates the substrate specificity and stability of the catalytic unit in the tetrameric complex (Bibby and Litchfield, 2005). Additionally, an intron-less CKII α pseudo-gene (CK2 α P) can be expressed in mammalian cells and has been shown to be associated with some cancers (Ortega et al., 2014; Wirkner et al., 1992).

CKII regulates many essential cellular processes including cell growth and proliferation, apoptosis, cell cycle regulation, DNA damage response, transcription, translation, chaperone-mediated protein folding, WNT signaling, mitotic spindle checkpoint, and circadian rhythm; with many of these processes being deregulated in cancers (reviewed in (Chua et al., 2017b; Rabalski et al., 2016)). There is an ample evidence of CKII being involved in the pathogenesis of cancer. It has been shown to function as an oncogene when overexpressed in mice (Landesman-Bollag et al., 2001; Ortega et al., 2014). The expression of CKII is often aberrant in cancers and increasing evidence suggests that CKII is often overexpressed and is linked to poor prognosis. However, in certain tumors (breast, ovarian and pancreatic cancer) CKII genes have been shown to be under-expressed, while in others over-expression was shown to correlate with high patient survival (e.g. lung adenocarcinoma) (Ortega et al., 2014). This indicates a strong degree of context dependency when assigning a role for CKII in cancer development.

In the case of HPV-associated cancer, clear evidence of CKII overexpression has not been reported; however, mRNA expression analysis using Oncomine analysis showed that all CKII transcripts were upregulated in cervical cancer (Chua et al., 2017a). In addition, CKII activity was shown to be higher (~ 3 fold)

in HPV-positive cervical cancer cell lines (SiHa, HeLa) and primary cell lines immortalized with HPV-16, compared with HPV-negative primary keratinocytes (Tugizov et al., 2005). Furthermore, sphere-forming cells (SFCs; cervical cancer stem-like cells) of the HeLa cell line were shown to have higher levels of CKII α compared with the parental cells, and inhibition of CKII α using apigenin inhibited the self-renewal capacity of these HeLa-derived SFCs, suggesting a potential use for CKII inhibitors in cervical cancer therapy (Piazza et al., 2006). In addition, CKII α activity was shown to be required for efficient replication of different HPV types, however, this was rather linked to the phosphorylation-dependent stability of the E1 protein (Piirsoo et al., 2019). Nevertheless, these findings strongly indicate CKII inhibition as a potential therapeutic approach to HPV-induced diseases.

Various cell-permeable CKII inhibitors have been developed. Some of the more widely-studied of these are TBB, Quinalizarin, hematein, TBCA, CIGB 300, DRB, apigenin, DMAT, Emodin and TF; and among them only CX-4945 and CIGB 300 have made it into clinical trials (Chua et al., 2017b; Ortega et al., 2014).

CIGB 300, is a synthetic chimera, consisting of a 9-mer cyclic peptide (P15 peptide, CWMSPRHLGTC), identified in a phage-display library screen as binding to the HPV 16E7 peptide (28LNDSSSEEEDEI38), conjugated via β -alanine to the HIV Tat cell-penetrating peptide (48GRKKRRQRRRPPQ68). CIGB 300 inhibited CKII mediated HPV-16 E7 phosphorylation and induced apoptosis in cell cultures and halted tumor growth in mice models (Perea et al., 2008; Perea et al., 2004; Perera et al., 2008). The first human clinical trial included 31 women with cervical cancer. CIGB 300 was administered intralesionally at four increasing doses over five consecutive days, and 75% of patients showed significant lesion reduction with minimal side effects even with the highest dose. Furthermore, 19% patients had a full histological regression and 48% patients (HPV DNA-positive) were HPV DNA-negative at the end of the trial (Solares et al., 2009). Further studies are ongoing with phase II where CIGB 300 in combination with chemoradiotherapy showed a response rate of 75% in 6 months, compared with 50% with chemoradiotherapy alone in 12 months (Perea et al., 2018).

CX-4945 (Silmitasertib), is a selective ATP-competitive inhibitor for CKII α and CKII α' catalytic subunits with an IC₅₀ of 1nmol/L *in vitro*. It exhibits a broad spectrum of antiproliferative activity and induces activation of caspase-3 and caspase-7 in normal cells (Pagano et al., 2004; Pierre et al., 2011; Siddiqui-Jain et al., 2010). Recently, CX-4945 was also shown to inhibit replication of some HPV types (HPV-18, -11 and -5) (Piirsoo et al., 2019). Furthermore, CX-4945 is in clinical trials for hematological and solid carcinoma treatments [Clinicaltrials.gov - NCT02128282 (Cholangiocarcinoma, phase 2), NCT03904862 (Medulloblastoma, phase 2) and NCT03897036 (Basal cell carcinoma, phase 1)]. Taken together, these studies on CKII inhibitors suggest targeting CKII could be a plausible therapy in HPV infection and HPV-induced cancers.

MMPs and HPV mediated carcinogenesis

Matrix metalloproteases are a family of zinc-dependent endopeptidases and play crucial roles in various physiological processes including tissue remodeling, organ development and regulation of inflammatory processes (Klein and Bischoff, 2011; Loffek et al., 2011). Human MMPs are classified as soluble and membrane-anchored (Klein and Bischoff, 2011; Loffek et al., 2011). Soluble MMPs contain an amino-terminal secretion leader sequence and they include collagenases (MMP1, MMP8, MMP13), stromelysins (MMP3, MMP10, MMP11), matrilysins (MMP7, MMP26), the metalloelastase (MMP12) and gelatinases (MMP2, MMP9). The membrane-anchored MMPs (membrane type MMPs, MT-MMPs) (MMP14, MMP15, MMP16, MMP17, MMP24, MMP25) contain a COOH-terminal trans-membrane domain linked to a cytoplasmic tail (Jablonska-Trypuc et al., 2016; Klein and Bischoff, 2011).

In physiological conditions, expression of MMP is generally low in most tissues and are induced or up-regulated only during tissue remodeling processes. In these processes, growth factors, hormones or cytokines trigger signaling molecules that, in turn, activate transcription factors, such as Activator Protein (AP)-1, ETS (E26 transformation-specific or E-twenty-six), Specificity protein (Sp)-1 and/or Nuclear Factor-kappa B (Gilet et al., 2015; Lin et al., 2017; Yan et al., 2015). Further, these activation pathways depend on cell types and are influenced by cell-to-cell contacts and cell-ECM interactions (Klein and Bischoff, 2011; Loffek et al., 2011; Rohani and Parks, 2015). The activity of soluble MMPs is kept under control by them being secreted as latent zymogens (proMMPs), which are converted to the active form around their producing cells, allowing a spatial control of MMP function. Furthermore, active soluble MMPs are regulated by endogenous inhibitors including serum globulin and Tissue Inhibitors of MMPs (TIMPs). TIMPs are expressed in most tissues and inhibit MMP activity by binding in their amino-terminal to the catalytic site of MMPs in 1:1 molar stoichiometry. Till date four TIMPs (TIMP 1-4) has been identified and have broad range of specificity in inhibiting MMPs (Brew and Nagase, 2010).

MT-MMPs are also synthesized as latent zymogens (proMT-MMPs) in cells, where they are converted to active MT-MMPs by the pro-hormone convertase

furin and then are expressed on the cell surface in a complex with TIMPs. The number of active MT-MMP molecules on the plasma membrane is regulated both by inhibitory activity of TIMP and autolysis of MT-MMPs in absence of TIMPs (Itoh, 2015). Thus, in normal homeostasis, the activity of MMPs is tightly regulated both at transcriptional and post-transcriptional level, however, in case of inflammatory diseases or cancers the regulation of MMPs is often perturbed leading to abnormal expression of MMPs and/or deregulation of their function (Folgueras et al., 2004; Yadav et al., 2014).

MMPs play a major role in tumour progression, by digesting molecules involved in mediating cell-to-cell or cell-to-ECM adhesion (e.g. cadherins or integrins), allowing tumour cells to detach from the ECM and adjacent cells. Further, MMPs degrade the basement membranes and the ECM, allowing a route for cancer cell migration and tissue invasion. These actions together allows the tumour cells to infiltrate in to the adjoining blood and lymph vessels leading to metastasis (Folgueras et al., 2004; Yadav et al., 2014). In addition, MMPs also play a significant role in tumour-associated angiogenesis by remodeling basement membrane of blood vessels and perivascular ECM. While, certain MMPs can also exert anti-tumour properties for example MMP8 expression was shown to be associated with increased survival in tongue cancer patients (Korpi et al., 2008).

In the case of development and progression of cervical cancer, various members of the MMP family including MMP-2, MMP-9 or MT1-MMP have been shown to be upregulated both at the RNA and protein level in high-grade CIN compared to normal cervix or low-grade CIN. Furthermore, upregulation of MMP1, MT1-MMP, MMP2, MMP9 has been shown to be due to expression of HPV E6 and E7 oncoproteins in various cellular models of cervical cancer (Kaewprag et al., 2013; Srivastava et al., 2017; Wang et al., 2014; Zhai et al., 2005; Zhu et al., 2015), which has also been linked molecularly to activation of AKT signaling which has been shown to activate transcription factors leading to *mmp* gene expression (Menges et al., 2006; Srivastava et al., 2017).

Novel targets of HPV E7

Most of the functions of HPV E7 proteins are due to interactions with cellular proteins as discussed earlier. There are numerous E7 interactions with host cell protein identified by various approaches (Poirson et al., 2017; Rozenblatt-Rosen et al., 2012; White and Howley, 2013), however, several of these proteins' function in HPV biology is yet to be identified. As most of these novel interactions are not delineated based on PTMs of E7 and in search of scrutinizing such targets to specifically identify targets dependent on phosphorylation of E7, a proteomic analysis using phosphorylated CR2 region of HPV-16 E7 described in this thesis identified, Vangl1 – planar cell polarity protein – as a novel HPV E7 interacting partner.

Wnt/PCP (Planar cell polarity) and cancer

Cell polarity is a fundamental characteristic of multicellular organisms which leads to correct orientation of cells both internally and with respect to one another. It refers to the localized distribution of cell polarity regulatory proteins within a cell. Most of our understanding of cell polarity derives from studies on invertebrates - *Drosophila melanogaster* and *Caenorhabditis elegans*. Cellular polarity module can be divided into two main types – (i) apico-basal polarity (ABP) defined by the orientation of cells perpendicular to the basement membrane and (ii) planar cell polarity (Wnt/PCP) defined by the orientation of cells parallel to the basement membrane or orthogonal to the apico-basal polarity (McCaffrey and Macara, 2011).

Wnt/PCP signaling is defined by asymmetric distribution of core proteins complexes within individual cells and this asymmetry is propagated across the tissue through intercellular protein-protein interactions (Devenport, 2014). The core Wnt/PCR components include, the seven-pass transmembrane receptor Frizzled (*Dorsophila* – Fz/mammalian Fzd), the atypical cadherin Flamigo (Fmi/Celsr), and the intercellular scaffolds Dishevelled (Dsh/Dvl) and Diego localize to the distal membrane, while the proximal membrane is marked by Fmi, the transmembrane tetraspanin scaffold Van Gogh (Vang/Vangl) and the intracellular scaffold Prickle (Pk). The asymmetric distribution of these core PCP protein complexes within cells are established by intracellular antagonism

between opposing complexes (Axelrod, 2001; Jenny et al., 2005; Tree et al., 2002; Warrington et al., 2017) (Figure 11A). And intercellular interaction between Wnt/PCP complexes on adjacent cells transmit this asymmetry to the neighboring cells, establishing propagation of asymmetry across many cell distances and demonstrating global signals to locally polarized cellular behavior (Chen et al., 2008; Strutt and Strutt, 2008; Wu and Mlodzik, 2008).

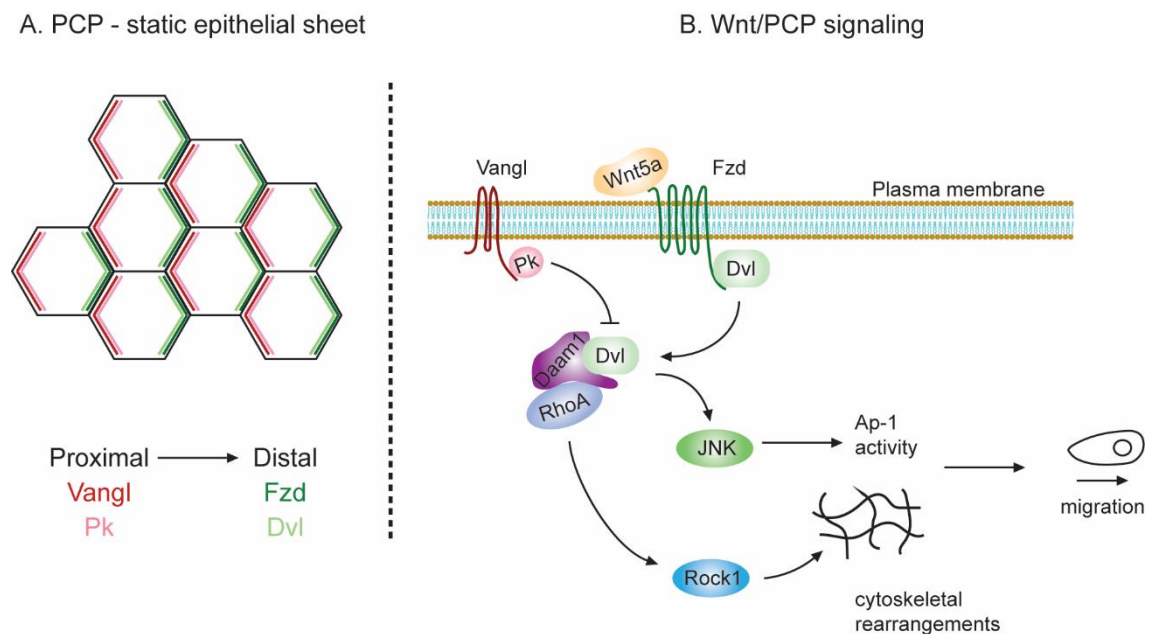


Figure 11. Wnt/PCP signaling in cancer cell migration.

(A). Planar cell polarity (orthogonal to the apical-basal axis) is governed by establishment of asymmetric distribution of Wnt/PCP protein complexes within a cell via intracellular antagonism between opposing complexes. (B). Activation of Wnt/PCP signaling occurs by noncanonical Wnt ligand (eg Wnt5a or Wnt11) binding to the Fzd receptor at the plasma membrane, which recruits and activates Dvl. The downstream effector components such as Rho family GTPases and JNK are then activated by Dvl, leading to modulation of actin cytoskeleton organization and promoting cellular motility. Adapted from (VanderVorst et al., 2018)

The molecular mechanisms governing the intracellular mutual antagonism are not completely clear, however, it has been suggested that post-translational modifications and directed protein trafficking play a major role in establishing and enforcing this asymmetry. Studies in *Drosophila* and mammalian models have suggested that the newly synthesized Wnt/PCP components are selectively trafficked to proximal or distal regions (Carvajal-Gonzalez et al., 2015; Matis et

al., 2014; Merte et al., 2010; Wansleebe et al., 2010) and these components are further regulated by post-translational modification such as ubiquitination providing local regulation at distal and proximal membranes establishing asymmetric localization, suggesting local negative regulation of PCP components essential for establishing global polarity within an epithelial tissue (Cho et al., 2015; Daulat et al., 2012; Kelly et al., 2016; Strutt et al., 2013). Further, in context of a motile cell, the antagonistic crosstalk between the opposing Wnt/PCP components both within a single cell and on adjacent cells, enables the context and tissue-specific cytoskeletal arrangements through the activation of downstream effectors. Wnt/PCP signaling is activated by non-canonical Wnt ligand (eg. Wnt5a or Wnt 11) binding to Fzd receptor at the plasma membrane, which recruits and activates Dvl. The downstream effector components such as Rho family GTPases and JNK are then activated by Dvl, leading to modulation of actin cytoskeleton organization and promoting cellular motility (Wald et al., 2017a) (Figure 11B).

Wnt/PCP is responsible for diverse events including proper embryonic development and structuring of multicellular tissues. In mouse embryo, proper neural tube closure (Kibar et al., 2001; Murdoch et al., 2001) and orientation of mouse hairs towards the posterior of the animal (Devenport and Fuchs, 2008) are some examples of the role of PCP in development and loss of proper PCP leads to various defects and disorders related to developmental processes. Mutations in core Wnt/PCP components such as Vangl1, Vangl2, Dvl1 and Dvl2, Fmi, and Fzd3 and Fzd6 have all been linked to neural tube defects (Kibar et al., 2007; Wang et al., 2006). While, in the case of cancer, reactivation of developmental pathways leads to tumour initiation and progression highlighting the connection between PCP signaling and cancer (Humphries and Mlodzik, 2018). Indeed, deregulation of core Wnt/PCP components has been reported to promote cell migration, invasion or metastasis in breast (Anastas et al., 2012; Pukrop et al., 2006), brain (Wald et al., 2017b), ovarian (Asad et al., 2014), prostate (Uysal-Onganer et al., 2010), gastric (Kurayoshi et al., 2006), and colorectal cancers (Ueno et al., 2008). Thus, contribution of Wnt/PCP signaling to progression of various tumours indicate that dysregulation of this pathway may be a feature common to tumor initiation and malignancy.

Van Gogh-like proteins - Vangl1 and Vangl2

Van Gogh-like proteins (Vangl1, Vangl2) are human orthologs of *Drosophila* Van Gogh (Vang/Stbm). It is a multi-pass transmembrane protein with cytoplasmic N-terminal with clusters of serine/threonine, and cytoplasmic C-terminal PBM motif (diagrammatic representation is shown in Figure 12) (Bastock et al., 2003; Wolff and Rubin, 1998). In *Drosophila* and in mammals, as discussed earlier, Vangl proteins are core components of Wnt/PCP pathway playing a major role in asymmetrical distribution across the cell and maintaining cell polarity critical for tissue morphogenesis (VanderVorst et al., 2018).

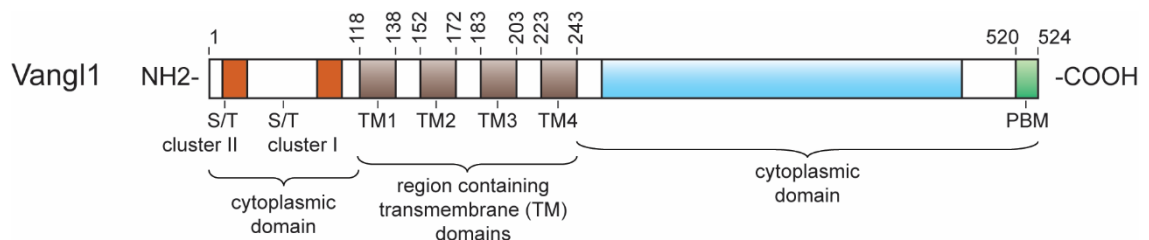


Figure 12. Diagrammatic representation of Vangl1 protein.

The Vangl1/2 proteins are cell surface proteins containing four transmembrane domains and intracellular amino- and carboxy-terminal domains. The N-terminal domain contains two serine-rich regions. The C-terminal domain contains protein-protein interaction motifs and conserved plasma membrane targeting motif.

Vangl proteins can also form homo- and hetero-oligomers and the process does not require N- or C-terminus cytoplasmic regions (Belotti et al., 2012). Vangl1 and Vangl2 appear nearly identical in their sequence and functions. Although alterations in Vangl2 result in more severe developmental defects (Song et al., 2010; Torban et al., 2008), suggesting a more central role for this form in early tissue morphogenesis, both proteins have been implicated in tumor progression.

Further, Vangl1/2 has also been implicated in apico-basal polarity. Scribble, a part of the complex that defines the basolateral domain and prevents expansion of the apical domain (reviewed in (Humbert et al., 2006)), interacts with Vangls (Anastas et al., 2012; Kallay et al., 2006) and functions as PCP effector in *Drosophila* (Courbard et al., 2009). Recently, in mouse models, Par-3 apical protein was shown to rescue Vangl1 localization defect caused by partial

knockdown of Scrib1, suggesting Scrib1 affecting Vangl1 localization indirectly through Par-3 (Kharfallah et al., 2017). These studies suggest that an intricate link between different polarity modules exists within and between cells that regulate the spatial position and direction of a cell in the tissue and dysregulation in any of the components or mutations would result in defects in cellular polarity as often seen in developmental defects or cancer.

Role of Vangl1 in cancer

Vang1 has been shown to be implicated in various cancers including head and neck squamous cell carcinoma (Lee et al., 2009), colon cancer (Lee et al., 2004), breast cancer (Anastas et al., 2012; Luga et al., 2012) and glioblastoma (Wald et al., 2017b). Anastas et al. showed that knockdown of Vang1 reduces migration of breast cancer cells and Vangl1 is localized in the leading edge of lamellipodia forming a complex containing scribble in wound healing assays. However, this complex was not detected in tightly packed non-transformed mammary cells, indicating altered localization of Vangl signaling contributes to breast cancer cell motility (Anastas et al., 2012). Further it was shown that treatment with exosome-containing conditioned media from mouse L-cell fibroblasts stimulated breast cancer cell motility dependent on Vangl1 and other PCP proteins (Luga et al., 2012). Downregulation of Vangl1 was shown to reduce proliferation (Yagyu et al., 2002) and invasion in hepatocellular carcinoma (Cetin et al., 2015). In a mouse squamous cell tumour model, Vangl1 was shown to enhance invasion and migration of mouse squamous cancer cells promoting pulmonary metastasis (Lee et al., 2009). In addition, Vangl1 was shown to enhance cell proliferation, migration and invasion of gastric cancer cells and enhance AP-1 dependent transcriptional activity (Ryu et al., 2010). Further, in case of bladder cancer, circular RNA of Vangl1 (circ-Vangl1) was shown to be highly expressed, negatively regulating miR-605-3p, and contributing to Vangl1 expression leading to cell proliferation, migration and invasion of bladder cancer cells (Zeng et al., 2019).

All these studies suggest a strong association of Vangl1 in tumour initiation and progression, however, a role in the case of cervical cancer has not been established till date.

Non-receptor protein tyrosine phosphatases - PTPN14 and PTPN21

Like many other PTMs, phosphorylation and de-phosphorylation of proteins is one of the most studied modifications in proteins, responsible for regulation of several cellular signaling. Proteins on tyrosine residues can be phosphorylated by protein tyrosine kinases (PTKs) and can be dephosphorylated by protein tyrosine phosphatases (PTPs). PTPs are known to regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, cell adhesion and oncogenic transformation (Tonks, 2006).

The first PTP to be identified was PTP1B in 1988 (Tonks et al., 1988), since then 125 PTPs have been identified in the human genome, which constitutes an enzyme superfamily divided into – the classical PTPs, dual-specificity PTPs and the low molecular weight PTPs (Alonso and Pulido, 2016a, b; Tonks, 2013). Classical PTPs are further divided into receptor and non-receptor PTPs. The PTPs have one or two catalytic domains with a conserved motif containing catalytic cystine - [(I/V)HCXAGXXR(S/T)G] – essential for removal of a phosphate group from a phospho-tyrosine residue (Tonks, 2013). PTPN14 (Pez or PTP36 or PTPD2) and PTPN21 (PTPD1 or PTPRL10) are members of classical, non-receptor PTPs family and contains N-terminal FERM (4.1 protein, ezrin, radixin, and moesin) domain, similar to cytoskeletal-associated proteins, which is a modular structure that mediates interactions with the plasma membrane and C-terminal phosphatase domain (Figure 13) (Tonks, 2006).

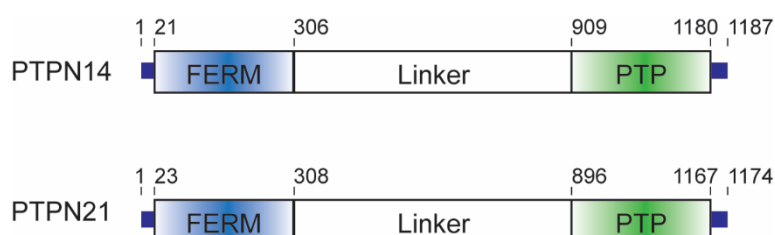


Figure 13. Schemantic representation of domains in PTPN14 and PTPN21.

PTPN14 and PTPN21 contains N-terminal FERM (4.1 protein, ezrin, radixin, and moesin) domain connected to C-terminal catalytic phosphatase (PTP) domain by an unstructured central linker region.

PTPN14 has been implicated as a cancer gene and has been frequently found mutated in colorectal cancer, pancreatic adenocarcinomas, basal cell

carcinomas and relapsed neuroblastomas (Bonilla et al., 2016; Wang et al., 2004). PTPN14 has been shown to inhibit oncogenic function of YAP (Yes associated protein) and other members of Hippo signaling pathway (Liu et al., 2013; Wang et al., 2012; Wilson et al., 2014). Furthermore, PTPN14 can decrease cell motility via dephosphorylation of β -catenin at adherens junctions (Wadham et al., 2003). Using a phospho-proteomic approach, PTPN14 was also shown to dephosphorylate p130 Crk-associated substrate (p130Cas) at tyrosine residue 128 in colorectal cancer cells, implicated in cell migration, anchorage-independent growth and increased activation of AKT (Zhang et al., 2013). In a breast cancer model, PTPN14 was shown to negatively regulate proteins involved in intracellular trafficking, such as RIN1 (Ras and Rab interactor 1) and protein kinase C- δ (PKRCD), thereby preventing metastasis by reducing the intracellular trafficking of both soluble and membrane-bound proteins (Belle et al., 2015). Interestingly, PTPN14 was identified as a potential cellular target of E7 proteins derived from multiple HPV types (Rozenblatt-Rosen et al., 2012; White et al., 2012). Subsequently, two groups including ours demonstrated independently that PTPN14 is a degradation target of high-risk E7 proteins (Szalmas et al., 2017; White et al., 2016). More recently, PTPN14 degradation was shown to perturb keratinocyte differentiation and potentially contributing to high-risk HPV E7-mediated oncogenic activity (Hatterschide et al., 2019).

PTPN21

PTPN21 was first isolated from human skeletal muscle in 1994 as a substrate and binding partner for the proto-oncogene SRC tyrosine kinase (Moller et al., 1994). PTPN21 also has a splice variant consisting only the catalytic domain lacking the SRC-binding region (Tokuchi et al., 1999). Kinesin-like protein KIFIC was shown to interact with PTPN21 FERM domain, suggesting a potential role of PTPN21 in the retrograde transport of Golgi-derived vesicles to the ER membrane (Dorner et al., 1998). PTPN21 has also been shown to interact with the cytoplasmic tyrosine protein kinase BMX, resulting in enhanced BMX kinase activity and STAT3 signaling (Jui et al., 2000). Further, A-kinase anchor protein AKAP121 was shown to bind and redistribute PTPN21 from the cytoplasm to mitochondria and inhibit SRC dependent EGF signaling (Cardone et al., 2004).

In addition, PTPN21 is able to bind to focal adhesion kinase (FAK), a scaffold protein enriched at adhesion plaques, promoting cell migration. Catalytic activities of PTPN21 and SRC were both required for EGF-induced FAK autophosphorylation and downstream signaling, suggesting PTPN21 plays a role in cell adhesion and migration (Carlucci et al., 2008).

PTPN21 has been implicated in bladder cancer, with levels of PTPN21 shown to be positively correlated with the grading and invasive potential of these tumours (Carlucci et al., 2010). Upon EGF stimulation, PTPN21 was shown to be recruited to endosomal vesicles, mediated by interaction with endosomal kinesin, KIF16B, which modulates receptor recycling at plasma membrane and downregulation of PTPN21 was shown to inhibit EGFR-ERK signaling affecting cell scattering and migration of bladder cancer cells (Carlucci et al., 2010). Using a genome-wide expression analysis in normal and cisplatin-resistant HeLa cells, it was demonstrated that PTPN21 was consistently upregulated in resistant cells and knockdown of PTPN21 sensitized the resistant HeLa cells to reverse the acquired chemoresistance, suggesting inhibition of PTPN21 as a potential target in case of cisplatin chemoresistance and thereby enhance efficacy of chemotherapy (Wu et al., 2010).

Thesis Aims

Aim 1. To determine the role of E7 phospho-acceptor site in maintenance of cell transformation

Despite, the role of CKII phospho-acceptor site in HPV lifecycle and transformation assays has been well established, there is little information on its role in maintenance of transformed phenotype. To address this question, I decided to genome edit CKII phospho-acceptor site in E7 in cervical cancer derived cells using CRISPR/Cas9 and HDR technology. Once a cellular model is established, then, to study effects on transformed phenotype.

Aim 2. To identify novel targets of HPV-E7 phospho-acceptor site.

HPV E7 is a multi-functional protein. It interacts with many different host cellular proteins to bring about range of different cellular functions towards viral life cycle and transformation. As previously described, CKII phosphorylation of E7 and its interaction dependent upon phosphorylation are important for these cellular functions, however, there is limited information on interactors of E7 dependent in CKII phosphorylation – thus to identify novel interactors of E7 dependent upon CKII phosphorylation of E7 is next aim.

Aim 3. Characterization of role of novel E7 interacting partner PTPN21

Recently, PTPN21 was identified as a binding partner of HPV E7s using proteomic screening approaches, suggesting a potential role of PTPN21 in HPV biology and carcinogenesis, however, the role of this interaction and its functional significance has not been characterized, therefore, I aimed to characterize the role of PTPN21/E7 interaction.

Results

Section I

The HPV-18 E7 CKII phospho acceptor site is required for maintaining the transformed phenotype of cervical tumour-derived cells

Mutation of HPV-18 E7 wild type to S32A/S34A abolishes phosphorylation by casein kinase II (CKII)

It was shown in the late 1980s that HPV-16 E7 was phosphorylated at residues S31 and S32 by CKII, and the equivalent residues S32 and S34 in HPV-18 were suggested to be likewise phosphorylated by CKII, based on protein sequence alignments (Firzlaff et al., 1989). In order to confirm the phosphorylation sites in HPV-18 E7, a series of *in vitro* phosphorylation assays were performed on purified GST-18 E7, using commercially available purified CKII and ^{32}P - γATP . To identify the specific phospho-acceptor residues in HPV-18 E7, single and double amino acid substitutions within the putative CKII phospho-acceptor site were also generated. The results obtained are shown in Figure 14 and demonstrate that both S32 and S34 are phosphorylated by CKII.

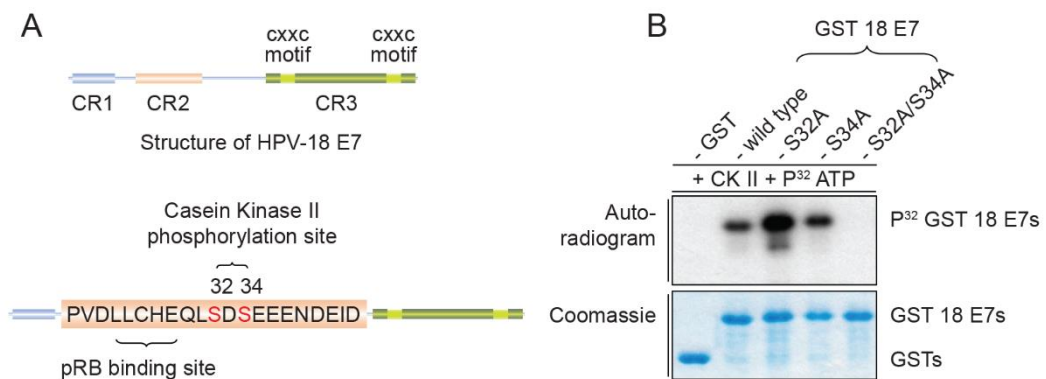


Figure 14. Mutation of HPV-18 E7 wild type to S32A/S34A abolishes phosphorylation by casein kinase II (CKII).

(A) Schematic representation of HPV-18 E7 conserved regions (CR), the pRB binding site and CKII phosphorylation site in the CR2 domain. (B) *In vitro* phosphorylation of GST HPV-18 E7 fusion proteins. Purified GST 18E7 wild type and mutants, as indicated, were incubated with CKII and $\gamma\text{P}^{32}\text{ATP}$. After extensive washing, bound proteins were subjected to SDS-PAGE and

autoradiographic analysis. The upper panel represents the autoradiogram and the lower panel represents the Coomassie Blue-stained gel.

Interestingly, the single S32A mutation appears to increase the overall level of E7 phosphorylation, although the reasons for this are unclear. However, mutation of both of these residues completely abolishes CKII phosphorylation of E7, confirming that these are the only CKII phospho-acceptor sites in HPV-18 E7. Taken together, these results demonstrate that both S32 and S34 are phosphorylated by CKII.

C4-1 cells are dependent on continued expression of E6 and E7 for growth and proliferation

The current genome editing techniques rely on an endogenous homologous DNA recombination (HDR) repair event, which is a less likely event than a non-homologous end-joining (NHEJ) repair event in mammalian cells (Guirouilh-Barbat et al., 2004). Therefore, in order to reduce the need for several genome editing steps to obtain a desired homozygous mutation in the HPV E7 gene in cervical cancer cells, the C4-1 cell line, which has only a single copy of HPV-18 DNA integrated into the host genome (Schneider-Gadicke and Schwarz, 1986), was chosen for this study.

Prior to performing genome editing it was first necessary to confirm that C4-1 cells were indeed still dependent upon the continued expression of E6 and E7 for maintenance of the transformed phenotype. To do this, the cells were transfected with siRNAs targeting HPV-18 E6 and 18 E6/E7, and after 72hrs the numbers of cells remaining were counted. The siRNA-transfected cells were also analysed for the levels of p53 and pRB using Western blotting. The results, together with the microscopic appearance of the cells, are shown in Figure 15, where it can be seen that C4-1 cells are indeed still dependent upon continued E6/E7 expression for maintenance of cell survival, in agreement with similar studies done in other HPV-positive cervical tumour-derived cell lines (Butz et al., 2003; Yamato et al., 2008; Yoshinouchi et al., 2003).

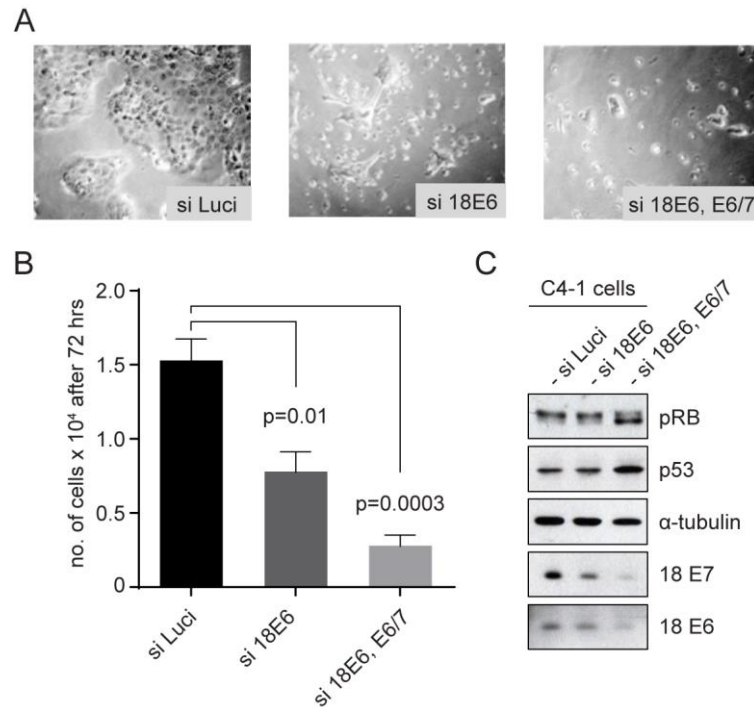


Figure 15. C4-1 cells are dependent on continued expression of E6 and E7 for growth and proliferation.

(A) Micrograph of the cells after 72h of siRNA treatment. C4-1 cells were transfected with si-Luciferase (si-Luci), si-HPV18 E6, si-HPV18E6 and E6/7 together, using RNAimax transfection reagent. (B) After 72h incubation, cells were counted to assess the remaining cell number and the graph shows the quantification after the different siRNA transfections. An unpaired t-test was used to compare mean cell number in siRNA transfections compared with siLuci, and p-values of the test are indicated over the bar graph. (C) Western blots showing levels of pRB, p53, α -tubulin, 18E7 and 18E6 after transfection with the indicated siRNAs.

Genome editing E7 wild type to S32A/S34A in C4-1 cells

To edit the E7 wild-type phospho-acceptor site in C4-1 cells to S32A/S34A, two guide RNAs were designed, aimed at creating a double-strand DNA (dsDNA) break close to the CKII phospho-acceptor site, plus a single stranded DNA oligonucleotide (ssODN) donor with the desired mutation, flanked by a 100 base-pair consensus to the HPV-18 genomic sequence, for the HDR repair event. These are depicted in Figure 16A and 18A. The ssODN donor DNA was also designed to insert a new HgaI restriction site in E7 to facilitate the screening of clones.

The efficiency of the guide RNAs was first confirmed using the surveyor nuclease assay in C4-1 cells, to analyze their ability to cut the target DNA sequence. As

in Figure 17), the cells were analysed for the S32A/S34A double mutation in the CKII phospho-acceptor site, by HgaI digestion of the 18E7 PCR amplicon (3 out of 408 screened) and then by DNA sequencing, whereby two such clones were ultimately identified (Figure 18B and 18C).

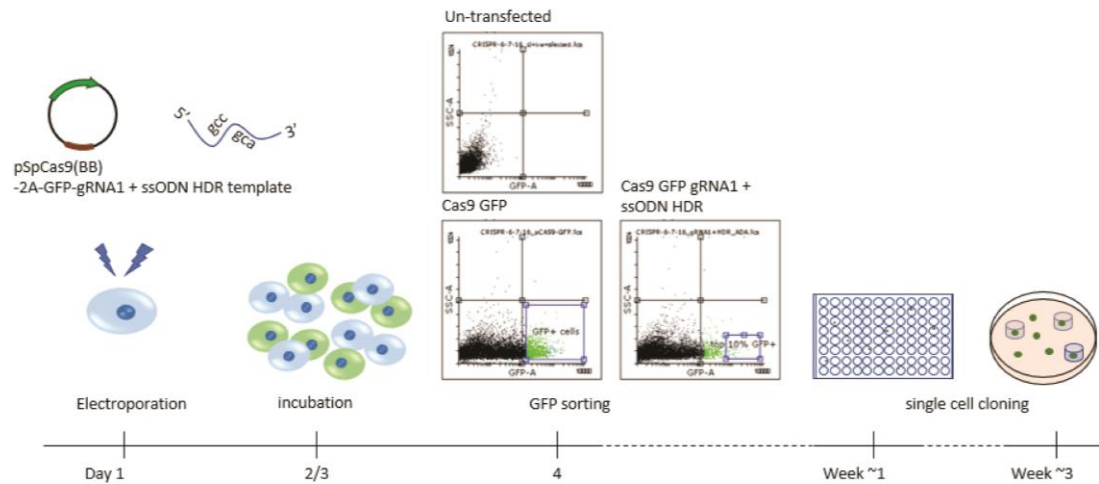


Figure 17. Schematic representation of strategic protocol for CRISPR/Cas9 genome editing HPV-18 E7 S32A/S34A in C4-1 cells.

Day 1: Cloned gRNA construct, together with single stranded DNA oligonucleotide donor (ssODN), was electroporated into C4-1 cells and incubated for 72 hours. Day 2/3: incubation period. Day 4: FACS sorting for GFP-positive cells, the top 10% of cells were sorted and plated in limited dilution in 10 cm dishes or 96 well plates for isolation of single cell colonies. Weeks 1-3: Screening of single cell colonies using PCR amplification, restriction digestion and Sanger sequencing.

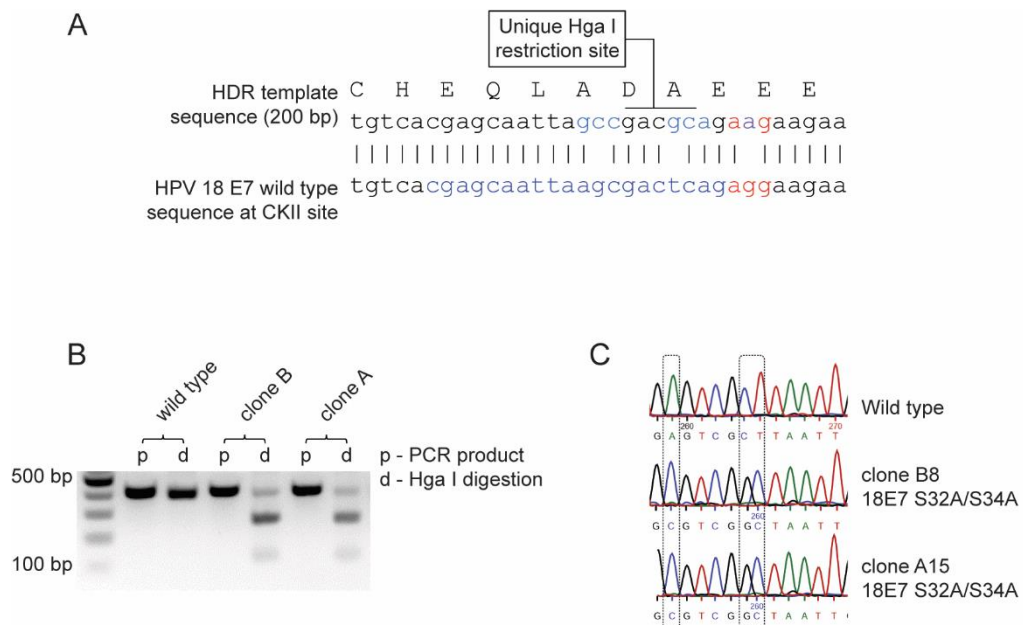


Figure 18. Design of HDR repair template for genome editing CKII site in HPV-18 E7 and screening and validation of edited C4-1 clones.

(A) Design of single-stranded DNA oligonucleotide (ssODN) as donor template for homology-directed repair (HDR). The ssODN was designed as a 100bp homology arm, flanking the predicted double strand break site to change the serine 32 to alanine (agc to gcc) and the serine 34 to alanine (tca to gca). The mutagenesis was designed to insert a unique HgaI restriction site, to allow screening of the edited clones by genomic DNA isolation, PCR amplification of the edited region and restriction digestion. (B) Representative agarose gel electrophoresis of Hga I digestion of 18E7 PCR amplicons from wild type and mutant clones. (C) Validation of the genome editing with Sanger sequencing.

CKII site mutant cells are less proliferative in low nutrient conditions

In order to fully characterize the CKII mutant clones obtained, first the growth rates of the cells were analysed in normal and low nutrient conditions. As can be seen from Figure 19, both the mutant clones grow somewhat more slowly than the wild type cells under normal culture conditions, and furthermore, the saturation densities are also consistently lower with the mutant cell lines. This is even more apparent when the cells are grown under conditions of low serum, where in 0.2% serum there is a dramatic decrease in the growth rate in the two mutant cell lines (Figure 19C and D). The growth curve in 0.2% serum (Figure 19C) reaches saturation density and drops after 4 days, possibly due to nutrient deprivation and cell death. When the assay is repeated, but replacing the medium after the third day (Figure 19D), it is clear that the wild type cells can

continue to proliferate while the mutant cells have greatly reduced rates of cell proliferation. These results suggest that mutation of the CKII phospho-acceptor sites in HPV-18 E7 in C4-1 cells has a markedly deleterious effect upon the ability of the cells to proliferate.

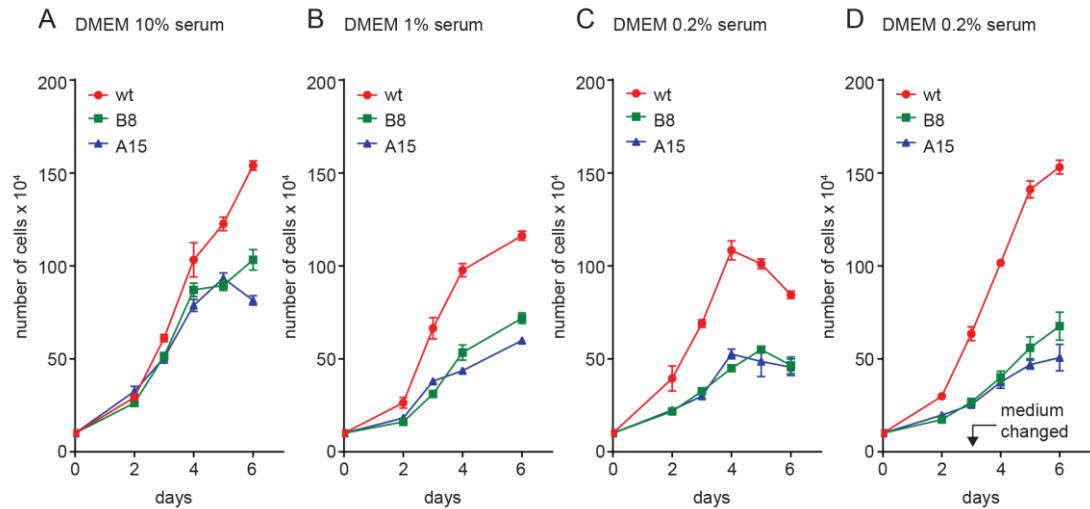


Figure 19. Growth of CKII site mutant cells is reduced in low nutrient conditions.

(A) C4-1 wild type and mutant lines were seeded at 3×10^5 cells per 60 mm petri dish, in DMEM with 10% fetal bovine serum and counted over a 6-day period. For determination of growth in low serum, the assay was repeated in DMEM with (B) 1% serum, (C) 0.2% serum and (D) 0.2% serum with medium replaced on the third day. The results show the mean of at least three independent assays and the error bars show standard deviations.

Levels of pocket proteins in CKII mutant cells and CKII phospho-acceptor site dependent interaction of E7 with pRB

Having shown that the mutant cells are less proliferative than the parental cells, we then analysed the levels of pocket proteins (pRB and p130) in these cell lines in comparison with the parental cell line. As can be seen from Figure 20, both the double mutant cell lines have slightly higher levels of pRB and p130 compared with the wild type line. Since previous studies had indicated that an intact CKII phospho-acceptor site can aid pocket protein recognition (Genovese et al., 2008; Jones et al., 1997), we also performed co-immunoprecipitation analyses between HPV-18 E7 and pRb in the wild type and mutant E7 cell lines. The results obtained are shown in Figure 20C and 20D, where it can be seen that there are lower levels of pRb complexed with E7 in the two mutant lines when compared with the wild type C4-1 cells. We also analysed the total levels

of expression of the E7 oncoprotein and, as can also be seen from Figures 20A and 20C, mutation of the CKII phospho-acceptor site had no deleterious effects upon the levels of E7 protein. In addition, the levels of p53 were also unchanged, suggesting that the levels of E6 expression were similarly unaffected. These results demonstrate that mutation of the E7 CKII phospho-acceptor site has a modest effect upon overall levels of pocket protein expression, as would be expected from previous structural studies (Chemes et al., 2010; Dick and Dyson, 2002; Genovese et al., 2008; Jones et al., 1997; Singh et al., 2005), however whether such changes are sufficient to account for the defects in cell proliferation remains to be determined.

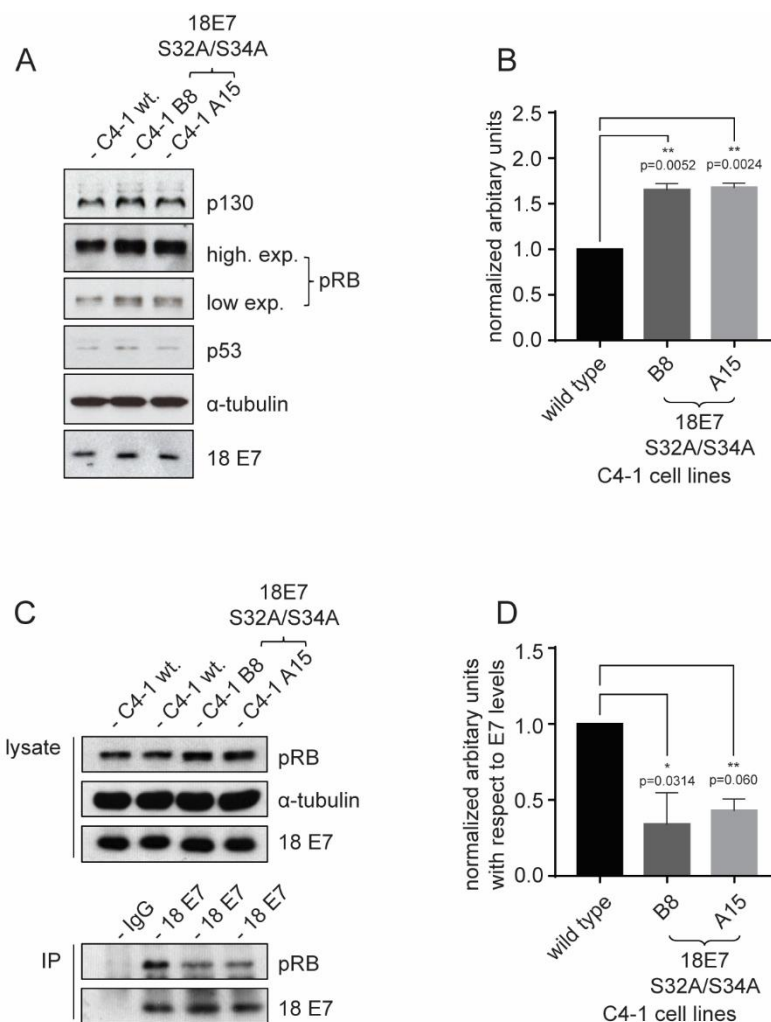


Figure 20. Steady-state levels of HPV-18 E7 and pocket proteins in the C4-1 cell lines.

(A) Western blot analysis of total cell extracts obtained from the wild type and mutant C4-1 lines. These were probed for p130, pRb, p53, HPV-18 E7, and α-tubulin was used as a loading control.

(B) Quantification of levels of pRB normalized with respect to α -tubulin shown as bars with the standard error of mean. Asterisks indicate the p-value of mean levels of pRB in mutant cells compared to wildtype cells. (C) Cells lysates from wildtype and CKII mutant C4-1 cells were immunoprecipitated with anti-HPV-18 E7 antibody overnight and then incubated with protein A beads for 90 minutes at 4°C. Immunoprecipitated complexes were then washed with lysis buffer and analysed by Western blotting for pRB. Mouse monoclonal anti GFP antibody was used as control IgG. The upper panels show the protein inputs for the immunoprecipitations, with α -tubulin serving as a loading control and the results of immunoprecipitation is shown in the lower two panels. (D) Quantification of the levels of pRB immunoprecipitated with respect to levels of E7 shown as bars with the standard error of mean. Asterisks indicate the p-value of mean levels of pRB immunoprecipitated in mutant cells compared with wildtype cells.

CKII mutant cells are less invasive in Matrigel invasion assay

We then proceeded to investigate the invasive capacity of the mutant clones using a matrigel invasion chamber assay. The cells were plated on the upper chamber in low serum and a chemo-attractant stimulus was provided in the lower chamber in the form of 2% calf serum. After 22hrs the lower chamber was fixed and stained and the number of migrating cells were counted. As can be seen from Figure 21, wild type C4-1 cells are very invasive, and migrate readily through the collagen matrix. In contrast, both mutant lines show defects in their invasive potential. Thus, the mutation within the HPV-18 E7 CKII phospho-acceptor site greatly decreases the ability of the cells to invade through a collagen matrix.

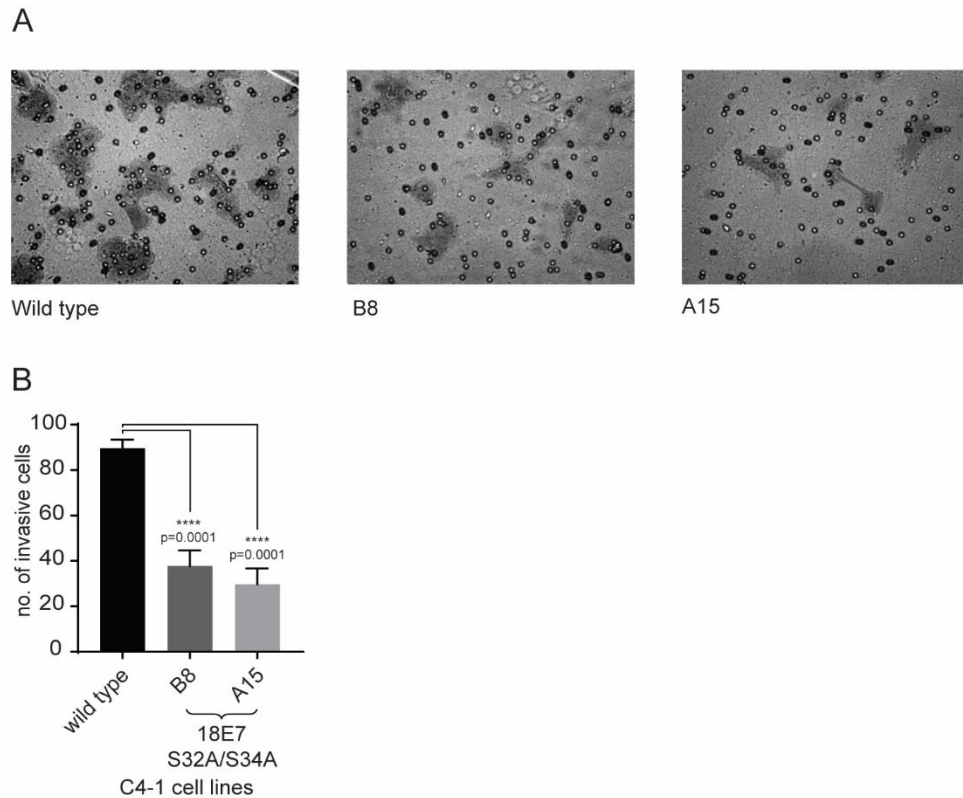


Figure 21. CKII site mutant C4-1 cells show defects in matrigel invasion.

Equal numbers of wild type and mutant C4-1 cell lines were seeded in the upper part of Matrigel invasion chambers, in DMEM without serum. DMEM with 2% of serum was used as a chemoattractant in the lower chamber. After 22h, cells that had invaded the lower chamber were fixed and stained with 0.5% Crystal Violet in 5% glutaraldehyde for 10 mins. (A) Representative micrographs showing invasive cells from each cell line as indicated. (B) Quantification of the mean number invasive cells shown as bars with the standard error of mean. Asterisks indicate the p-value of mean numbers of invasive cells, compared with the wild type cells.

Furthermore, when the CKII mutant cells were analysed for migratory abilities in a scratch wound healing assay in a 2D setting, it was rather surprising that they show no apparent differences in this assay (Figure 22), suggesting the defect of CKII mutant cells is particularly in ability to migrate in a 3D matrix, suggesting possible defects in the ability to invade through an extracellular matrix (ECM).

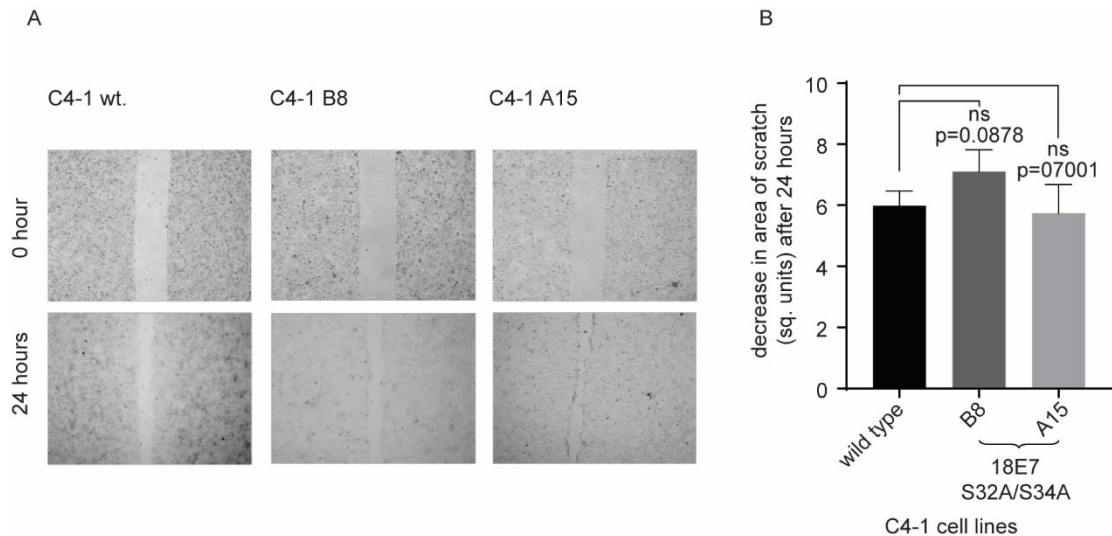


Figure 22. Scratch wound healing assay for migratory abilities of C4-1 cells.

(A) Confluent wild type and CKII mutant C4-1 cells were scratched with a sterile Artline p2 pipette tip. The cells were washed twice with PBS and photographed immediately and after 24 hours. (B) The decrease in area of the scratch was analysed and quantified using the Image J and Prism programs, is shown as bars with standard error of mean.

Defective invasive ability of CKII mutant cells links to downregulation of MMPs

To investigate whether the mutant cells' defective invasion potential was related to any change in their ability to hydrolyse the extracellular matrix, we concentrated conditioned media from the mutant cells and analysed their activity by Gelatin Zymography. As can be seen in Figure 23A, conditioned media from the mutant lines are unable to hydrolyse the gelatin gel, whereas that from the wildtype cells shows significant bands of gelatin hydrolysis. We then performed a series of western blot analyses on the conditioned medium to try and determine which matrix metalloproteases might be involved. As can be seen from Figure 23B and 24A and 24B, both MMP1 and MMP13 are significantly downregulated in the two mutant clones when compared with the wild type C4-1 cells. This appears to be highly specific, since there is no change in the levels of expression of MMP8 (Figure 24C).

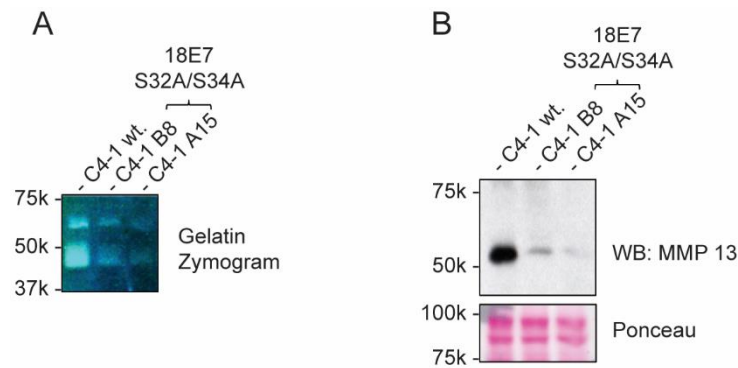


Figure 23. Defect in ability to hydrolyse gelatin linked to downregulation of MMP13 in CKII site mutant C4-1 cell lines.

(A) Confluent wild type and CKII site mutant C4-1 cell lines were incubated in a serum-free medium for 48 hours. The conditioned media from the cell lines were concentrated and equal amounts of protein run on non-reducing 10% SDS-PAGE containing 0.1% gelatin. The gel was then renatured and incubated overnight at 37°C for gelatin hydrolysis. After incubation, the gel was stained with Coomassie Blue to visualize the bands of hydrolyzed substrate. (B) Equal amounts of concentrated conditioned media from the indicated cell lines were analysed by western blotting for MMP13 (upper panel). The lower panel shows the Ponceau stain of the nitrocellulose to confirm equal protein loading.

To determine whether the defect in MMP secretion was a reflection of a slower growth rate in the clones, we repeated the assay but induced growth arrest in the wild type C4-1 cells with mitomycin C treatment for 48hrs. Secreted MMP levels were then analysed by western blotting and, as can be seen from Figure 25, there is no apparent difference in MMP levels in the growth arrested cells. These results indicate that the differences in MMP levels between the wild type and mutant C4-1 cells, are not due to differences in proliferation.

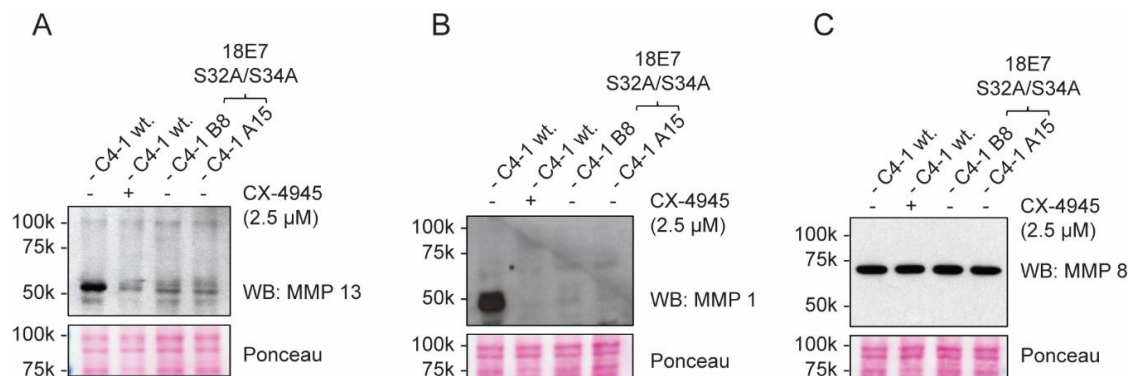


Figure 24. Matrix metalloproteases MMP 1 and MMP 13 are downregulated in CKII site mutant C4-1 cell lines.

Confluent wild type and CKII site mutant C4-1 cell lines were incubated in a serum-free medium for 48 hours. Additionally, a different set of wild type cells were treated with 2.5 μ M of CX-4945, as indicated, to specifically inhibit CKII activity. The conditioned media from the cell lines were concentrated and equal amounts of concentrated conditioned media from the indicated cell lines were analysed by western blotting for (A) MMP1, (B) MMP13 (C) MMP8 (upper panels). The lower panel shows the Ponceau stain of the nitrocellulose to confirm equal protein loading.

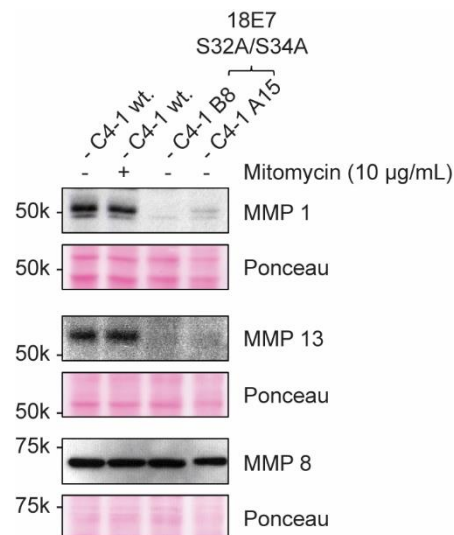


Figure 25. Higher levels of MMP1 and MMP13 in condition medium of C4-1 wild type are independent of cell proliferation.

Confluent cells, as indicated, were changed to serum free medium and incubated for 48 hours. Ten μ g/mL of mitomycin C was added to one of the wild-type C4-1 cells to inhibit cell proliferation. After 48 hours conditioned media from the cells were harvested, concentrated and analyzed by SDS PAGE and Western blotting to detect levels of indicated MMPs.

In order to determine whether the high levels of MMP1 and MMP13 expression are dependent upon CKII activity, we treated the wild type C4-1 cells with the highly specific CKII inhibitor CX-4945 and analysed the effects upon the protein levels of MMP1 and MMP13. As can be seen from Figure 24A and B, CX-4945 treatment of the wild type cells reduces the levels of MMP1 and MMP13 expression to levels similar to those seen in the two mutant lines. This suggests that active CKII phosphorylation of HPV-18 E7 is required for maintaining high levels of MMP protein expression in C4-1 cells.

All the above analyses were done on the C4-1 cells, but we wished to determine whether high levels of MMP secretion was specific to active CKII in HPV-positive cells. To do this we analysed the levels of secreted MMPs in HPV-16 positive CaSki cells and HPV-negative C33a cells in the presence and absence of CKII inhibitor. The results obtained are shown in Figure 26A and B and demonstrate that high levels of secreted MMP1 and MMP13 in CaSki cells are also dependent upon active CKII, whilst in C33a cells inhibition of CKII has no effect upon the levels of secreted MMPs.

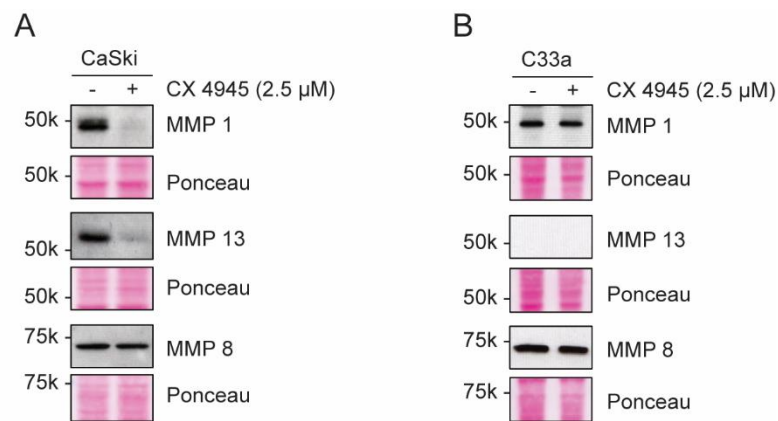


Figure 26. Matrix metalloproteases MMP 1 and MMP 13 are downregulated in CaSki but not in C33a cells after CKII inhibition.

Confluent HPV-16 positive, CaSki and HPV negative, C33a cells, were incubated in a serum-free medium for 48 hours either in presence or absence of 2.5 μM of CX-4945, as indicated. Then conditioned media from the cell lines were concentrated and equal amounts proteins were processed as before and analysed by western blotting for MMP13, MMP1 and MMP8 (upper panels), with the lower panels in each case showing the Ponceau stain of the nitrocellulose membranes.

Downregulation of MMP1/13 can be rescued in CKII mutant cells by exogenous expression of wild type HPV-18 E7

These results indicate a specific role for active CKII in regulating MMP levels in HPV positive cells. To verify this, we performed a rescue experiment where we overexpressed FLAG-tagged wild type HPV-18 E7 in the two mutant lines and examined the effects on MMP levels secreted by the cells. Figure 27A shows the levels of endogenous and overexpressed HPV-18 E7. As can be seen from Figure 27B, in cells expressing high levels of wildtype FLAG-tagged HPV-18 E7 the amounts of secreted MMP1 and MMP13 were increased, while no effect was

seen on the levels of MMP8 (Figure 27B). In order to determine whether E7 affected the total levels of MMP1 expression we also performed western blot analysis on total cell extracts derived from the wild type, mutant and E7 overexpressing cells. As can be seen from Figure 27C the intracellular levels of MMP1 are unaffected by mutation of the E7 CKII phospho-acceptor site and remain unchanged following overexpression of wild type E7.

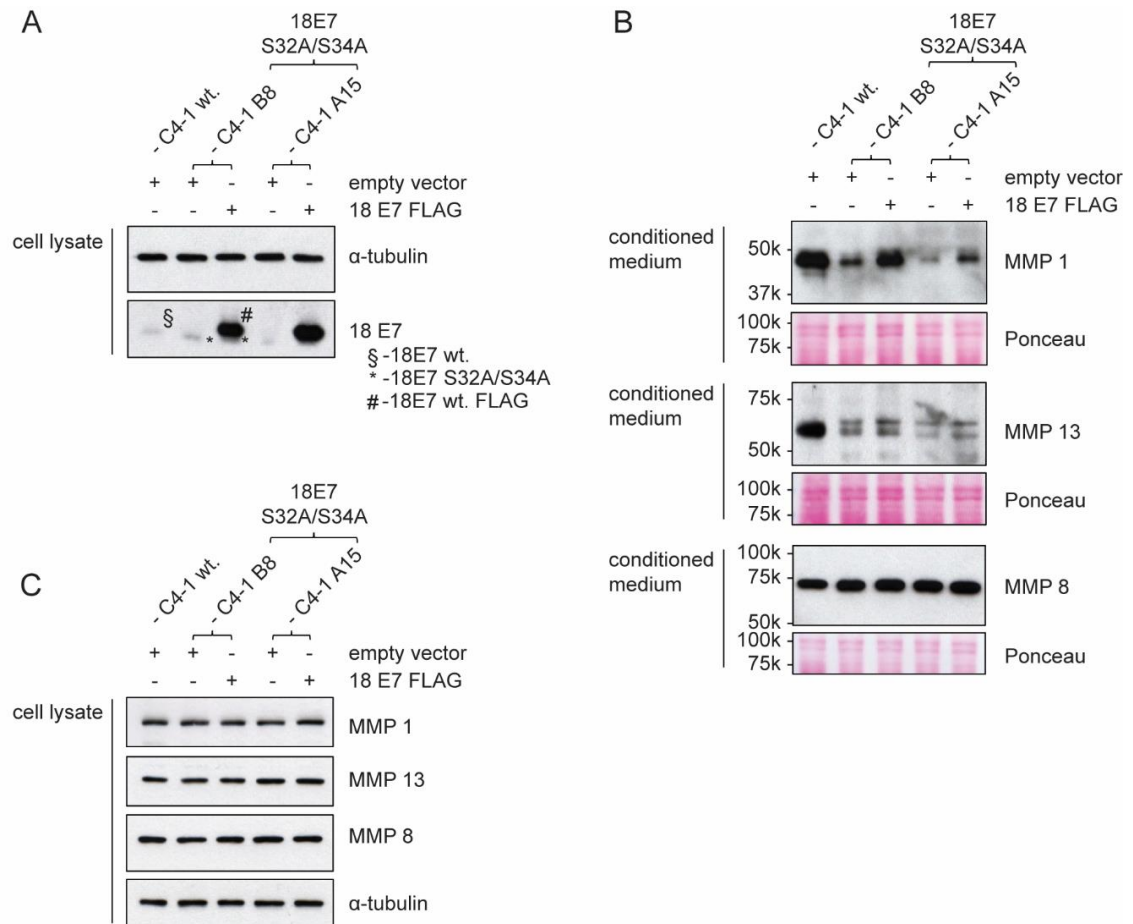


Figure 27. Exogenous expression of wild type HPV-18 E7 can rescue the downregulation of MMP 1 and MMP 13 in CKII site mutant cell lines.

Wild type C4-1 cells were transfected transiently with empty pCMV vector and mutant cell lines with pCMV vector expressing FLAG-tagged wild type HPV-18 E7. (A) The cells were then harvested directly in 2X sample buffer and analysed by SDS-PAGE and Western blotting for levels of 18E7 and α-tubulin. The positions of endogenous wild type E7 (§), the S32A/S34A mutant (*) and the wild type FLAG-tagged E7 (#) are also shown. (B) Concentrated conditioned medium from the cells was analysed by western blotting for MMP1, MMP13 and MMP8 (upper panels). The lower panels show the Ponceau stain of the nitrocellulose membranes to confirm equal levels of protein input. (C) Western blot analysis of total cell extracts for MMP1, MMP13 and MMP8 with α-tubulin used as the loading control.

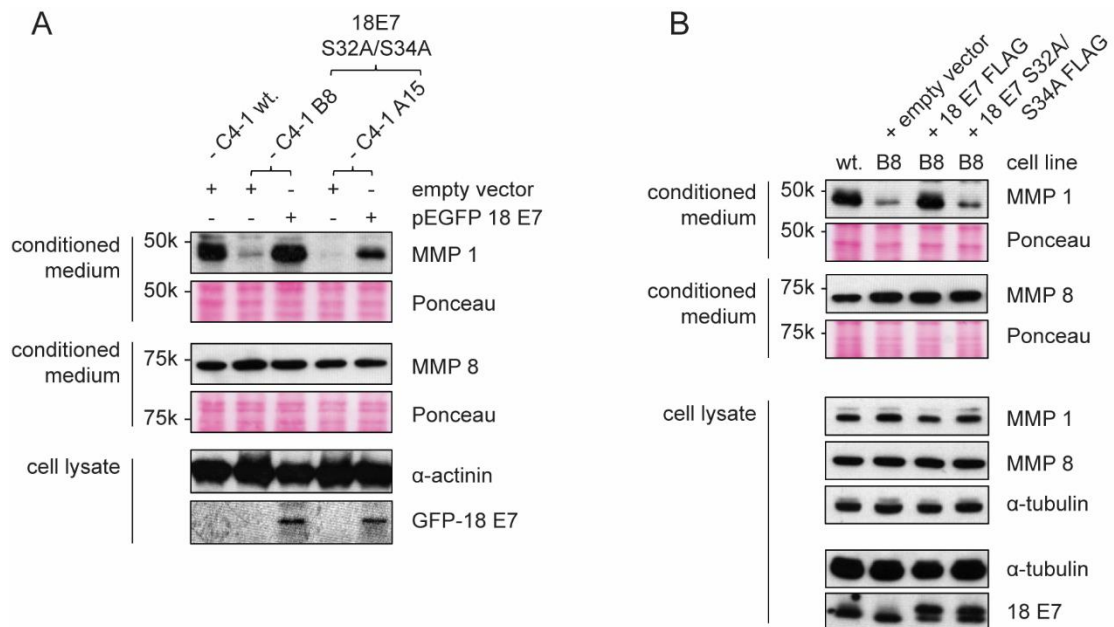


Figure 28. Stable expression of wild type HPV-18 E7 can rescue the downregulation of MMP 1 and is dependent on an intact phospho-acceptor site in E7.

(A) C4-1 cells were transfected with pEGFP 18E7 wildtype plasmid or empty vector as indicated and selected by FACS sorting and G418 selection to obtain cells stably expressing GFP-E7. The cells were analysed for MMP1 and MMP8 levels in the conditioned medium, and the expression of GFP-18E7 was ascertained in the cell lysate by western blotting. (B) C4-1 B8 CKII mutant cells were transfected with pCMV empty or pCMV:18E7 wildtype or pCMV:18E7 S32A/S34A mutant expressing plasmids, as indicated, and selected with G418 to obtain stable cell lines. The cells were then analysed for MMP secretion in the conditioned medium and the cell lysate by western blotting.

Since the rescue of the levels of secreted MMPs was only partial, which was most likely due to a low level of (approx. 20%) transfection efficiency, we proceeded to generate cells stably expressing wild type HPV-18 E7. To do this, the CKII mutant cells were transfected with plasmid expressing GFP-18E7 and selected by FACS sorting and subsequent G418 selection. The pooled cells were then analysed for the levels of secreted MMPs. As can be seen from Figure 28A, both of the CKII mutant polyclonal cell lines stably express GFP-18E7. Furthermore, in these rescued cells the conditioned medium has very similar levels of secreted MMP1 to that seen in the wild type C4-1 cells. To further verify that this rescue is due to phosphorylation of stably expressed wild type HPV-18 E7, cells stably expressing a C-terminal FLAG tagged wild type HPV-18 E7 and

the HPV-18 E7 S32A/S34A mutant were also obtained and analysed for MMP secretion. As can be seen from Figure 28B, despite similar levels of wild type and mutant HPV-18 E7 S32A/S34A expression, wild type levels of MMP1 secretion were only observed in the cells expressing the wild type HPV-18 E7 protein. These results demonstrate that an intact E7 CKII phospho-acceptor site plays an essential role in ensuring high levels of MMP secretion in a HPV-18-positive cervical tumour-derived cell line.

Downregulation of MMP1 and MMP13 in C4-1 and CaSki cells downregulates invasive ability

Having identified MMP1 and MMP13 as being upregulated in a CKII dependent manner in HPV-positive cells, we then asked if knockdown of MMP1 and MMP13 would have any deleterious affect upon the invasive ability of the wild type cells. To do this, we transfected siRNA against luciferase as control, or MMP1, or MMP13, or a combination of the two into wild type C4-1 cells, and after 48 hours pooled equal numbers of cells and performed a Matrigel invasion assay. As can be seen from Figure 29A-C, there is marked decrease in invasion upon knockdown of MMP13, little effect with MMP1 knockdown alone, but a marked synergistic affect when both MMP1 and MMP13 were removed. Similar results were obtained when the assay was performed in HPV-16 positive CaSki cells (Figure 29D-E).

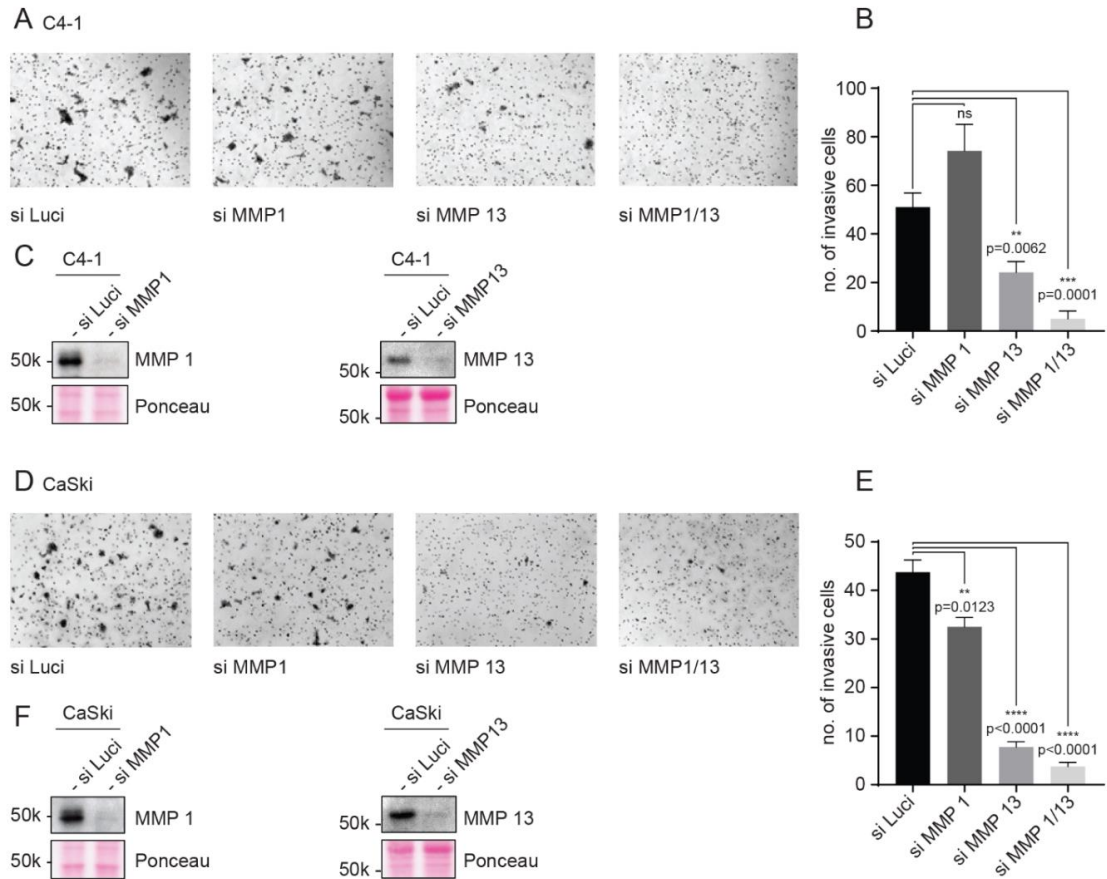


Figure 29. Knockdown of MMP1/13 abrogates invasion of C4-1 and CaSki cells.

C4-1 and CaSki cells were transfected with indicated siRNAs. After 48 hours, equal numbers of cells were seeded in the upper part of Matrigel invasion chambers, in DMEM without serum. DMEM with 10% serum was used as a chemoattractant in the lower chamber. After 22h, cells that had invaded the lower chamber were fixed and stained with 0.5% Crystal Violet in 5% glutaraldehyde for 10 mins. (A and D) Representative micrographs showing invasive cells from each siRNA treatment as indicated. (B and E) Quantification of the mean number of invasive cells per field shown as bars with the standard error of mean obtained from at least 4 separate fields. Asterisks indicate the p-value of mean numbers of invasive cells, compared with the wild type cells. (C and F) Western blot analysis of concentrated conditioned medium from indicated siRNA treatments for MMP1 and MMP13.

Taken together, these results demonstrate that the loss of CKII phosphorylation of HPV-18 E7 in cells derived from a cervical cancer, has diverse inhibitory effects upon the ability of the cells to proliferate and to invade a collagen matrix, and the defect in the ability to invade is at least in part due to downregulation of MMP1/13.

CKII phospho-acceptor site in high-risk E7 is important for sustained activation of AKT and expression of extracellular MMP1/13

We were next interested in understanding the molecular basis for this defect. Previous studies had implicated the ability of E7 to activate AKT signaling as being in part responsible for promoting invasive behavior (Srivastava et al., 2017). However, no studies have been done to investigate whether CKII phosphorylation of E7 can lead to activation of AKT, and whether this in turn might be responsible for the increased levels of MMP secretion. To investigate these possibilities we first analysed the ability of wild type and CKII site mutants of HPV-16 and HPV-18 E7 to activate AKT in a transient assay in HEK293 cells.

In order to first verify the levels of E7 phosphorylation in these assays, we generated a phospho-specific antibody (refer Appendix I and Material Methods section for details) directed against the HPV-16 E7 CKII phospho-acceptor site. The characterization of this antibody is shown in Figure 30, where it can be seen that it only reacts against the phosphorylated form of HPV-16 E7. After 24 hours, the transfected 293 cells were serum-starved for 16 hours and then harvested and analysed by western blotting. As can be seen from Figure 31A, only the wild type E7 is capable of inducing AKT phosphorylation. Interestingly, the phospho-specific antibody detects the wild type E7 but fails to react against the CKII phospho-site mutant. These results confirm that the wild type E7 protein is phosphorylated to a significant degree, but that there is no phosphorylation of the double CKII phospho-site mutant of E7.

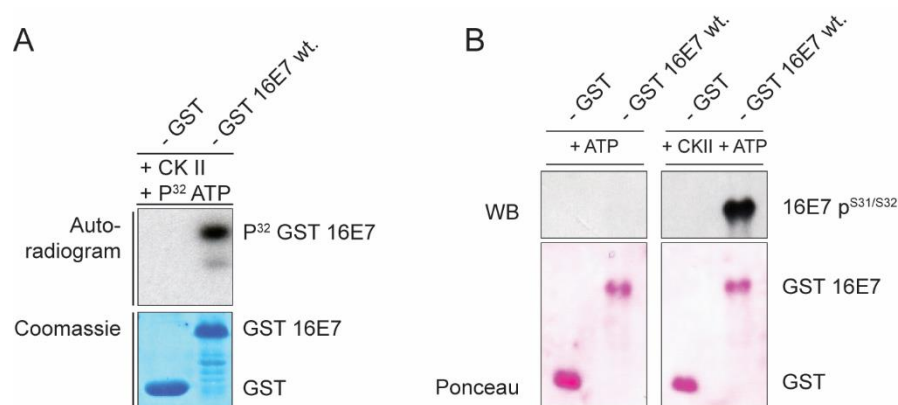


Figure 30. Validation of HPV-16 E7 phospho-specific antibody.

(A) *In vitro* phosphorylation of GST HPV-16 E7 fusion protein for cross-validation of phospho-specific antibody. Purified GST fusion proteins, as indicated, were incubated with CKII and γ [P³²] ATP. After extensive washing, bound proteins were subjected to SDS-PAGE and autoradiographic analysis. The upper panel represents the autoradiogram and the lower panel represents the Coomassie Blue-stained gel. (B) Purified GST fusion proteins, as indicated, were incubated either with or without CKII enzyme (NEB) in kinase buffer in the presence of ATP for 15 min at 30°C. After extensive washing with kinase wash buffer, GST fusion proteins were analysed by Western blotting for HPV-16 E7 phospho-specific antibody. Levels of GST fusion proteins are shown by Ponceau staining of the nitrocellulose membrane.

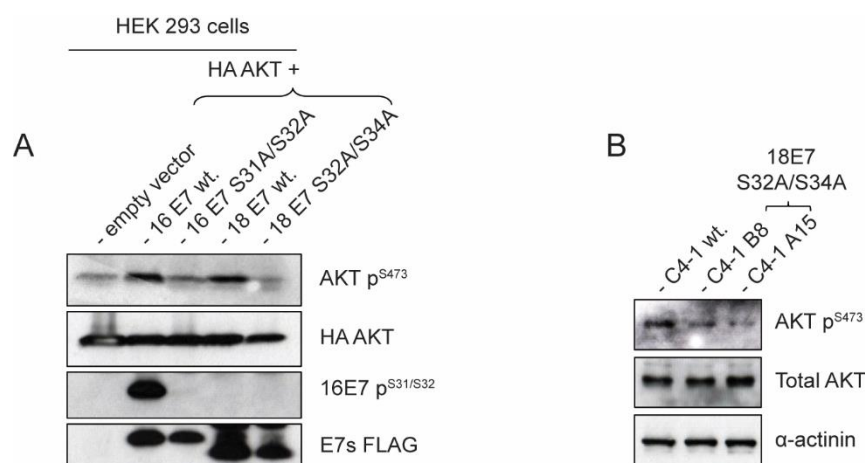


Figure 31. Activation of AKT is perturbed in the absence of CKII phosphorylation of E7.

(A) HEK 293 cells were transfected with HA-AKT together with HPV-16 E7 FLAG-HA, wildtype or HPV-16 E7 S31A/S32A, HPV-18 E7 FLAG, wildtype or HPV-18 E7 S32A/S34A or empty vector. After 24 hours, the cells were serum starved for 16 hours and harvested directly with SDS PAGE sample buffer and analysed by Western blotting for phospho-AKT, total AKT (HA), phospho-16E7 and total E7 (FLAG), as indicated. (B) Confluent C4-1 wildtype and CKII phospho-site mutant C4-1 cells were serum starved for 48 hours and total levels of AKT, p-AKT, and α -actinin was analysed by Western blotting.

Having found that an intact CKII phospho-acceptor site was required for the ability of E7 to increase AKT phosphorylation we then analysed the levels of AKT phosphorylation in the wild type and CKII phospho-site mutant C4-1 cells following serum starvation for 48 hours. The results in Figure 31B also confirm the requirement for an intact CKII phospho-acceptor site to obtain high levels of AKT phosphorylation.

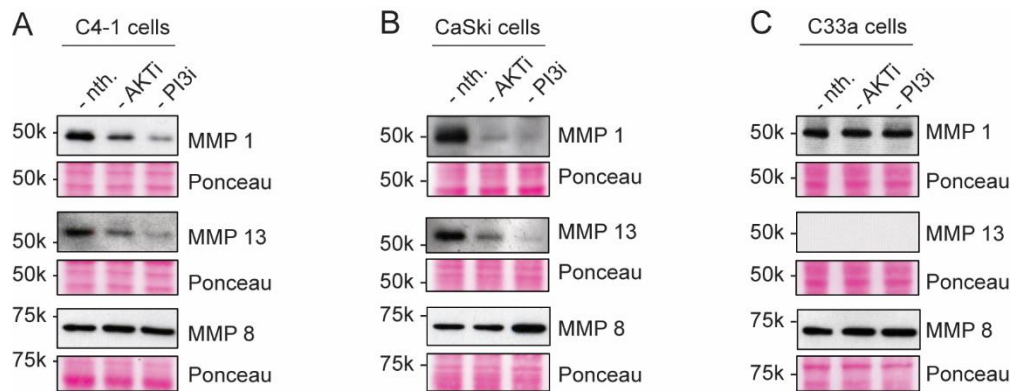


Figure 32. Inhibition of PI3/AKT pathway downregulates MMP1/13 in HPV-positive, C4-1 and CaSki cells but not in HPV-negative C33a cells.

Confluent C4-1 (A), CaSki (B) and C33a (C) cells were either treated with AKT inhibitor or PI3K inhibitor in serum-free medium for 48 hours. Conditioned medium from the treatments were analysed by Western blotting for MMP1, MMP13 and MMP8. In each case the lower panels show the Ponceau stain of the nitrocellulose membranes.

These results implicate AKT signaling in HPV-positive cells as being responsible for the high levels of MMP secretion. To investigate this further we analysed the levels of MMP1 and MMP13 secretion in HPV-18-positive C4-1 cells, HPV-16-positive CaSki cells and HPV-negative C33a cells following inhibition of AKT and PI3K using the specific AKT inhibitor (124005-Calbiochem) and PI3K inhibitor (LY294002). After 48 hours the supernatants were harvested and analysed by western blotting. As can be seen from Figure 32A-C, blocking AKT signaling in HPV-positive cells results in a marked decrease in the levels of secreted MMP1 and MMP13, whilst this has no effect in the HPV-negative cells. In agreement with results in Figures 24 and 26, which showed that CKII phosphorylation of E7 had no effect on MMP8, likewise blocking AKT signaling also had no effect on MMP8 secretion in HPV-positive cells. Taken together these results demonstrate that CKII phosphorylation of E7 promotes AKT activation, which in turn is responsible for increased MMP secretion and subsequent enhancement of invasive potential in HPV-positive cervical tumour-derived cells.

Section II

Planar cell polarity protein Vangl1 is targeted by Human Papillomavirus E7 oncoprotein

Proteomic analysis identifies Vangl1 as novel interactor of HPV-16 phospho-E7

In the previous section, we showed that CKII phosphorylation of E7 was important for maintenance of the transformed phenotype of cervical cancer derived cell lines. By using genome editing techniques, we showed that CKII phosphorylation of E7 was required for both cell proliferation and for optimum levels of MMP secretion thus contributing to cellular invasive abilities. These intriguing results from genome editing of the E7 CKII phospho-acceptor site, led us to look further for potential mechanistic explanations for these effects. We reasoned that most proteomic analyses that had been done previously had used E7 in asynchronously growing cells, where there was likely to be both phosphorylated and non-phosphorylated forms of E7. However, since phosphorylation has been shown to be so important for E7 function, we wished to try and identify phospho-specific interacting partners of E7. To do this, a proteomic approach was chosen. We synthesised short peptides corresponding to the CKII site in CR2 region of E7, either phosphorylated or non-phosphorylated, to act as bait in a pull-down assay to identify phosphorylation-dependent interactors. Also included were peptides corresponding to the CR2 region of the HPV-16 E7 N29S variant, which has an additional phospho-acceptor serine at position 29, and which was recently shown to have higher transforming abilities than the wild-type (Zine El Abidine et al., 2017). The biotinylated peptides used in this study are shown in Figure 33.

scrambled control peptide:	Biotin-RRLQRTVEQR
non-phospho-peptide:	Biotin-LQPETTDLYCYEQLSDSSEEEDEIDG
phospho-peptide:	Biotin-LQPETTDLYCYEQL pSDpSp SEEEDEIDG

Figure 33. Biotinylated peptide sequences used for pulldown assay and downstream proteomic analysis.

All peptides were synthesized in-house by the ICGEB Biotechnology Development facility. The scrambled peptide sequence is a stretch of 10 random amino acids, while non-phospho-peptide is CR2 region of HPV-16 E7 N29S variant from residues 15-39 and phospho-peptide is the with phosphorylated serine residues at position, S29, S31 and S32.

The peptides were conjugated to Streptavidin-coated magnetic sepharose beads and used in a pulldown assay with HaCaT cell extract. Mass spectroscopy analysis of the proteins pulled down by phospho- and nonphospho-peptides of HPV-16 E7 CR2 region revealed several different proteins, and the top 10 major proteins identified in a single experiment are shown in Table 1. As can be seen, pRB is identified in both cases, with the higher total number of pRb peptides being pulled down by the phosphorylated E7 peptide compared with the non-phosphorylated E7 peptide, supporting previous studies showing that negative charges or phosphorylation of the E7 CKII phospho-acceptor site enhances its binding to pRB and related pocket proteins (Chemes et al., 2010; Dick and Dyson, 2002; Singh et al., 2005). It is technically possible that the peptide could be phosphorylated by the HaCaT cell extract during the pull-down assays. However, if this was to occur it would result in no difference between the phospho-peptide and non-phospho-peptide in the pull-down assays. Since, we did observe such differences as it seems that there is still a significant degree of phosphorylation between the two peptides. Among several other precipitated proteins, including ribosomal proteins, keratins, splicing factors and others, Vangl1 was intriguing to note, as it was specifically precipitated by phospho-peptide of E7.

Table 1. Identification of phospho-specific interactors of HPV-16 E7.

Biotinylated peptides were conjugated with magnetic streptavidin beads and incubated with HaCaT cell extract. After 3X washing with the lysis buffer the beads were washed with detergent free lysis buffer. Buffer-free beads were then analysed for identification of bound proteins using the ICGEB MS facility. The top 10 hits of the proteomic analysis are shown (refer to Appendix II for complete list of identified proteins).

A. HPV-16E7 N29S phospho-peptide

Rank	Unique	Total	Description
	peptides	peptides	
1	6	28	RPL3, ribosomal protein L3
2	7	14	MEMO1, mediator of cell motility 1
3	5	13	RB1, retinoblastoma 1
4	4	6	VANGL1, VANGL planar cell polarity protein 1
5	3	10	RPS8, ribosomal protein S8
6	3	11	TUBB4A, tubulin, beta 4A class IVa
7	1	1	TUBB4B, tubulin, beta 4B class IVb
8	3	15	RPL10, ribosomal protein L10
9	3	13	RPL19, ribosomal protein L19
10	3	10	KRT5, keratin 5, type II

B. HPV-16E7 N29S non-phospho-peptide

Rank	Unique peptides	Total peptides	Description
1	7	34	MEMO1, mediator of cell motility 1
2	5	10	RB1, retinoblastoma 1
3	4	12	KRT14, keratin 14, type I
4	3	6	LUC7L2, C7orf55-LUC7L2 readthrough
5	4	8	KRT19, keratin 19, type I
6	3	5	RBM39, RNA binding motif protein 39
7	3	9	KRT5, keratin 5, type II
8	3	4	TUBA1B, tubulin, alpha 1b
9	2	4	RPL21, ribosomal protein L21
10	2	7	SRSF3, serine/arginine-rich splicing factor 3

To verify the proteomic analysis that suggested a potential interaction of E7 phospho-peptide with Vangl1, the peptide pulldown was analysed by western blot and probed for Vangl1. As can be seen from Figure 34A, Vangl1 was specifically detected bound to the phospho-peptide, while it was undetectable with the non-phosphorylated peptide. Again, the E7 CR2 phospho-peptide bound a higher amount of pRB than the non-phosphorylated E7 CR2 peptide, as expected. To determine whether this interaction could be mimicked by introducing a negative charge the phospho-serines were replaced with the acidic residue aspartic acid in order to act as a potential phospho-mimic. Surprisingly the interaction between the mutant E7 peptide and Vangl1 was almost undetectable, indicating that simple replacement of serine with aspartic acid is not sufficient to confer interaction with Vangl1 and that a true phosphorylation event is required (Figure 34B).

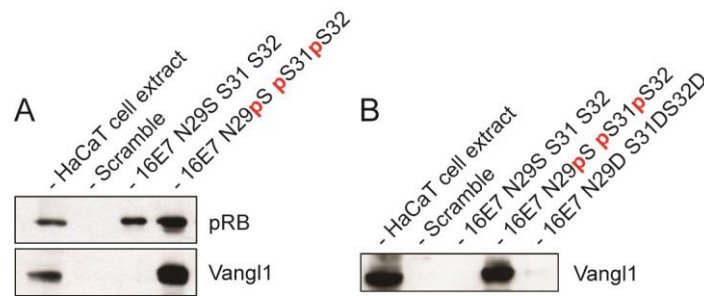


Figure 34. Validation of Vangl1 as a phospho-specific interactor of HPV-16 E7 phosphopeptide.

Biotinylated peptides, as indicated, were conjugated with magnetic streptavidin beads and incubated with HaCaT cell extracts for an hour at 4°C. After extensive washing with lysis buffer, the beads were mixed with 2X sample buffer and analysed with SDS PAGE and Western blotting for pRB and Vangl1.

To examine this interaction further, the next question was, does this interaction remain valid in terms of full-length E7 in transient overexpression assays? To do this, HEK 293 cells were transfected with FLAG-Vangl1 and C-terminally FLAG-HA tagged CMV 16E7 wild-type or S31A/S32A or N29S constructs. After 24 hours, cells were harvested in RIPA buffer and incubated with anti-HA conjugated agarose beads. After extensive washing, the immune-complexes were analysed by SDS-PAGE and Western blot, probed for Vangl1 with anti-FLAG antibody, for E7 with anti-HA antibody and for phosphorylated HPV-16 E7 with the phospho-specific antibody described in section I, Figure 30. As can be seen from Figure 35, the wild-type and N29S variant of E7 are phosphorylated, as detected by the phospho-specific antibody, and they specifically bind Vangl1, while 16E7 S31A/S32A is defective in pulling down similar amounts of Vangl1, suggesting that the interaction of Vangl1 and E7 is dependent on phosphorylation of E7 at the CKII site.

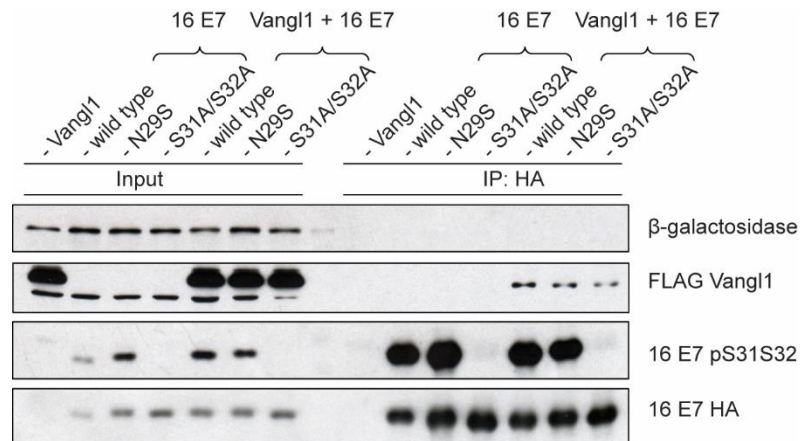


Figure 35. HPV-16 E7 interacts with Vangl1 *in vivo* and is dependent on phosphorylation of E7.

HEK 293 cells were transfected with FLAG-Vangl1, together with HPV-16 E7 FLAG-HA, wildtype or N29S or S31A/S32A or empty vector. β-galactosidase vector was used as transfection control. After 24 hours, the cells were lysed using RIPA buffer and incubated with anti-HA agarose beads for 90 minutes at 4°C. After incubation, cells were washed with E1a buffer and analysed by SDS PAGE and Western blotting for β-galactosidase, Vangl1 (anti-FLAG), 16 E7 (anti-HA) and phospho-16 E7 (anti-16E7pS31/S32) as indicated.

Further, we repeated the immunoprecipitation assay using CX4945, where addition of this specific CKII inhibitor strongly decreased the phosphorylation of E7 wildtype and N29S variant, and at the same time also greatly reduced the amount of Vangl1 co-immunoprecipitated with E7 (Figure 36). This again supports the notion that interaction between E7 and Vangl1 requires phosphorylation of E7 by CKII.

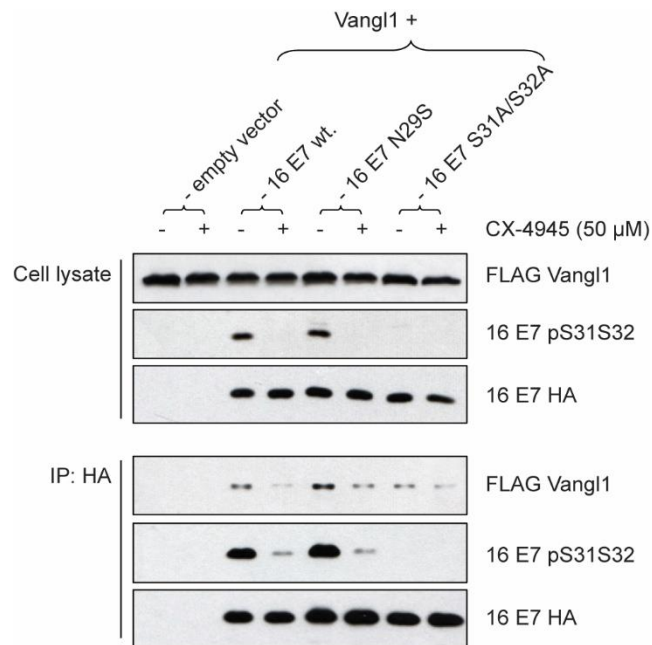


Figure 36. Vangl1 interaction with HPV-16 E7 is perturbed in presence of a specific CKII inhibitor.

HEK 293 cells were transfected with FLAG-Vangl1 together with HPV-16 E7 FLAG-HA, wildtype or N29S or S31A/S32A or empty vector as indicated in duplicate. β -galactosidase vector was used as transfection control. After 24 hours, duplicate transfections were treated, or not, with 50 μ M of CX-4945 for 5 hours. Cells were then lysed using RIPA buffer and incubated with anti-HA agarose beads for 90 minutes at 4°C. After incubation, cells were washed with E1a buffer and analysed by SDS PAGE and Western blotting for β -galactosidase, Vangl1 (anti-FLAG), 16 E7 (anti-HA) and phospho-16 E7 (anti-16E7pS31/S32) as indicated.

Interaction of Vangl1 with phospho-E7 is conserved between high-risk and low-risk types

Since previous studies have shown that, high-risk and low-risk E7 could be phosphorylated at equivalent residues in the CR2 region (Barbosa et al., 1990; Firzlaff et al., 1989), we asked if the interaction with Vangl1 was conserved between these HPV types. To do this, HEK 293 cells were transfected with FLAG-Vangl1 in combination with C-terminally FLAG-HA tagged, high-risk E7s (HPV-16 E7 or HPV-18 E7 or HPV-31 E7) or low-risk HPV-11 E7. Immunoprecipitation was carried out, as before, and analysed by Western blotting. The results shown in Figure 37 indicate that this interaction is conserved between both high-risk and low-risk HPV types and is dependent upon active CKII.

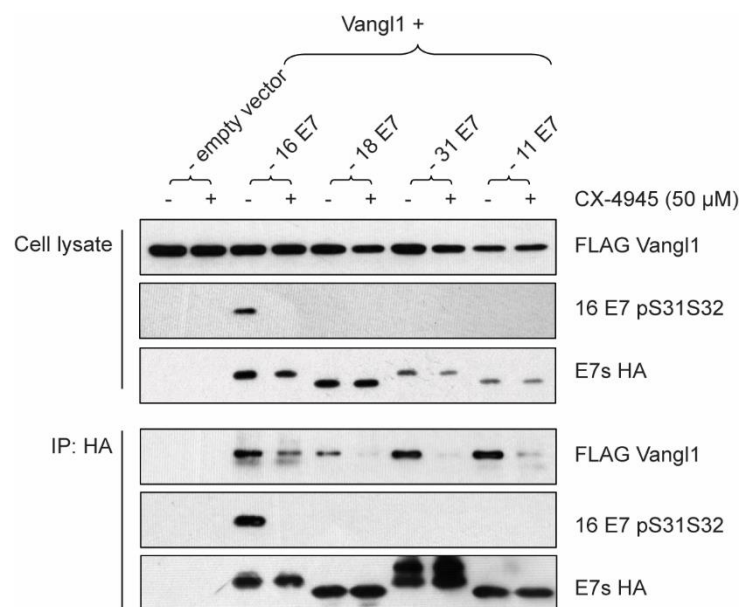


Figure 37. Vangl1 interaction with high- and low-risk E7s in a CKII phosphorylation dependent manner.

HEK 293 cells were transfected in duplicate with FLAG-Vangl1 together with C-terminally FLAG-HA tagged HPV-16, HPV-18, HPV-31 or HPV-11 E7s or empty vector, as indicated. β -galactosidase vector was used as transfection control. After 24 hours, one set of transfections was treated with 50 μ M of CX-4945 for 5 hours. Cells were then lysed using RIPA buffer and incubated with anti-HA agarose beads for 90 minutes at 4°C. After incubation, cells were washed with E1a buffer and analysed by SDS PAGE and Western blotting for β -galactosidase, Vangl1 (anti-FLAG), E7s (anti-HA) and phospho-16 E7 (anti-16E7pS31/S32) as indicated.

Vangl1-phospho E7 interaction is perturbed in genome edited C4-1 cells

Having shown that the Vangl1 interaction with phosphorylated E7 was highly conserved, it was interesting to know if this interaction might be perturbed in the genome edited HPV-18 E7-positive C4-1 cells described in section I, Figure 18C. To examine this, the wildtype and CKII mutant C4-1 cells were lysed using lysis buffer, the supernatant was collected by centrifugation and incubated with 2 μ g of anti-HPV-18 E7 antibody at 4°C overnight. The immune complexes were then incubated with protein-A agarose beads for 90 minutes and immunoprecipitated complexes were analysed by Western blotting. The results are shown in Figure 38, where we can see that, both the CKII mutant E7s in the genome edited cell lines bind less Vangl1 than the wild type E7 in the parental C4-1 cell line. Additionally, although pRb protein levels are higher in the CKII mutant cell lysates, less of it is immunoprecipitated than in wild type cell lysates.

Furthermore, the steady-state levels of Vangl1 are downregulated in genome edited cells compared with mutant cells, indicating that the wildtype E7 in the parental C4-1 cell might be playing a role in maintaining higher steady-state levels of Vangl1. Vangl1 has been implicated in several cancers, often mutated or amplified perturbing its function (Hatakeyama et al., 2014). In case of cervical cancer, mutations are not so common (refer Appendix III, cBioPortal: expression and mutation profile of Vangl1 and p53 in cervical cancer) suggesting E7 (CKII phosphorylation of E7) might be upregulating its expression as seen in wild type C4-1 cells compared to CRISPR edited CKII mutant cells. However, this potential modulation of Vangl1 upon E7 phosphorylation needs further investigation.

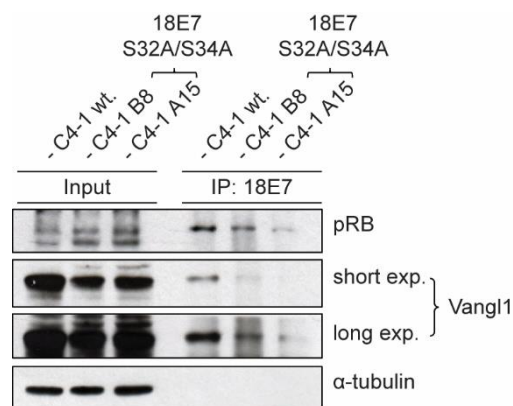


Figure 38. Vangl1-phospho E7 interaction is perturbed in genome edited C4-1 cells.

Cells lysates from wildtype and CKII mutant C4-1 cells were immunoprecipitated with anti-HPV-18 E7 antibody overnight and then incubated with protein A beads for 90 minutes at 4°C. Immunoprecipitated complexes were then washed with lysis buffer and analysed by SDS PAGE and Western blotting for pRB, Vangl1 and α-tubulin.

Vangl1 co-operates with HPV-16 E7 in cell transformation

Having found that the interaction of Vangl1 is conserved across several HPV E7s, and considering previous reports on the role of Vangl1 in development, cellular migration and invasion, we were interested in whether Vangl1 has any direct effects upon the transforming activity of HPV E7. To examine this, primary BRK cells were transfected with EJ-ras, with pJ4Q:HPV-16 E7 or Vangl1 or with pJ4Q:HPV-16 E7 and Vangl1. After 2 weeks of selection, the cells were fixed

and stained, and the number of colonies were counted. The results are shown in Figure 39, where it can be seen that both HPV-16 E7 and Vangl1 can transform primary BRK cells in cooperation with ras. Furthermore, the combination of Vangl1 and HPV-16 E7, in cooperation with ras, acts synergistically to dramatically increase the level of transformation of primary BRK cells, suggesting that the interaction with Vangl1 is potentially important in HPV-E7-mediated cellular transformation. The current assay shows that Vangl1 co-operates with E7 in transforming BRK cells, however, future studies will have to be supplemented with c-myc, Vangl1 and the E7 CKII mutant or CKII inhibitors for delineating the role of phosphorylation of E7 in the transforming ability in co-operation of Vangl1.

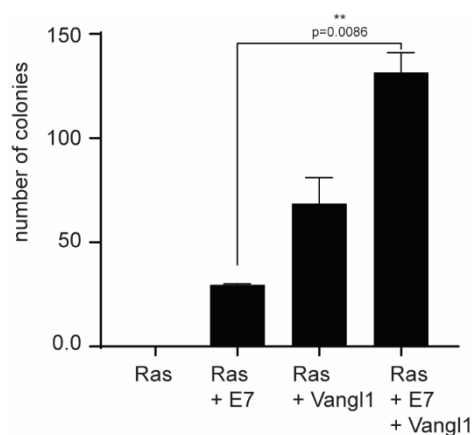


Figure 39. Vangl1 enhances BRK cell transformation ability of E7 in HPV-16 E7/EJ-ras transformation experiments.

Primary BRK cells were transfected with the indicated expression plasmids and subjected to selection with G418. After 2 to 3 weeks, the cells were fixed and stained with Giemsa stain and the numbers of colonies were counted. The results show the mean number of colonies with standard deviations as error bars.

Preliminary data

Previous studies have shown that Vangl1 is implicated in the invasive ability of various cancers, including breast cancer (Anastas et al., 2012), head and neck cancer (Lee et al., 2009) and hepatocellular carcinoma (Cetin et al., 2015; Yagyu et al., 2002). Vangl2, a paralogue of Vangl1 has been implicated in regulation of

MMP14, by decreasing its availability at the plasma membrane (Williams et al., 2012). To determine if there is any change in steady-levels of MMP14, a Western blot analysis was done with the wildtype and mutant C4-1 cells. The results in Figure 40 show that MMP14 levels are lower in CKII mutant cells, suggesting that the phospho-dependent interaction of Vangl1 might be involved in regulation of MMP14 and invasion. Indeed, downregulation of Vangl1 phenocopies defects in the invasive ability of CKII mutant cells, suggesting a link between this function of phosphorylation of E7. Downregulation of Vangl1 has been suggested to result in decrease in invasive ability of hepatocellular carcinoma cells (Cetin et al., 2015), whether the observed downregulation of Vangl1 in genome edited cells, is involved in decreasing invasive ability remains to be determined. Further details of the localization and distribution of Vangls, contributing PCP components, and MMPs (eg. MMP14) in relation to E7 phosphorylation would uncover the role of Vangl1 in modulating polarity and transformed phenotype in cervical cancer derived cells.

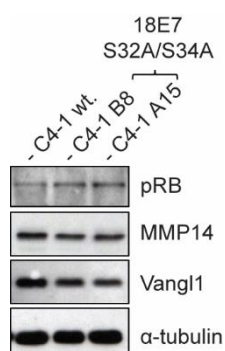


Figure 40. Steady-state levels of MMP14 in C4-1 cell lines.

Steady-state levels of MMP14 in C4-1 wild-type and mutant cells was analysed by harvesting indicated cells directly into 2X sample buffer and analysed by SDS PAGE and Western blotting for MMP14, Vangl1, pRB and α-tubulin.

Section III

PTPN21 is a degradation target of high-risk HPV E7

Previous studies using proteomic approaches identified PTPN14 and PTPN21 as potential cellular targets of E7 proteins derived from multiple HPV types (Rozenblatt-Rosen et al., 2012; White et al., 2012). Subsequently, two groups, including ours, demonstrated independently that PTPN14 is a degradation target of high-risk E7 proteins (Szalmas et al., 2017; White et al., 2016). While PTPN21, was identified in the same proteomic study as binding partner of HPV E7s neither of the groups discussed the role of this potential interaction. Here, we have begun to study the PTPN21 as a binding partner of HPV E7s and consequences of the binding in modulation of PTPN21 by HPV E7.

PTPN21 interacts with HPV E7s

To verify the interaction of PTPN21 with HPV E7 proteins that was indicated by proteomic approaches (Rozenblatt-Rosen et al., 2012; White et al., 2012), a GST pull-down assay using high-risk HPV-16 and HPV-18, and low-risk HPV-11 E7 with *in vitro* translated PTPN21 was carried out and analyzed by SDS-PAGE and autoradiography. As can be seen from Figure 41A, both high-risk and low-risk E7 interact with PTPN21, although to our surprise HPV-18 E7 interacts only weakly, whilst HPV-11 is as strong as HPV-16 E7. Further, to identify regions in E7 responsible for interaction, another set of GST pull-down assay was carried out with C-terminal and N-terminal halves of HPV-16 E7 and the results are shown in Figure 41B, where we can see that PTPN21 also binds to the E7 carboxy-terminal region.

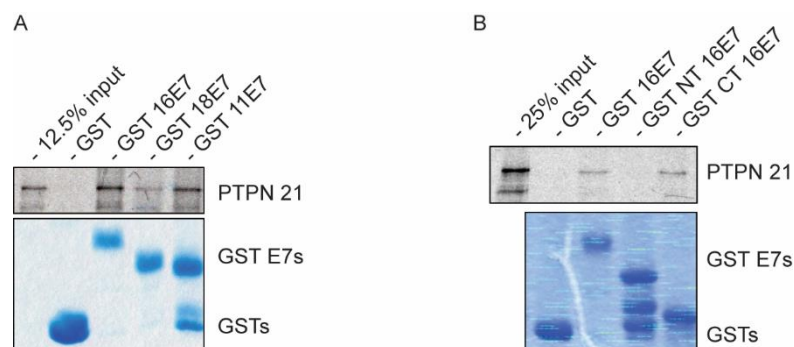


Figure 41. PTPN21 interacts with high-risk and low-risk HPV-E7s.

(A) *In vitro* translated, radiolabeled PTPN21 was incubated with GST, GST-16 E7, GST-18 E7 or GST-11 E7 fusion proteins bound to glutathione agarose beads, as indicated, at room temperature for 1 hour. After washing the beads, bound proteins were assessed by autoradiography (upper panel) and GST fusion proteins were visualized by Coomassie staining (lower panel). (B) *In vitro* translated, radiolabeled PTPN21 was incubated with GST, GST-16 E7, N-terminus GST-16 E7 or C-terminus GST-16 E7 fusion proteins bound to glutathione agarose beads as indicated at room temperature for 1 hour. After washing the beads, bound proteins were assessed by autoradiography (upper panel) and GST fusion proteins were visualized by Coomassie staining (lower panel).

Previous studies have shown that multiple residues within the C-terminus of E7 are important for potential protein-protein interactions and for their ability to contribute toward cell transformation, based on their surface exposure (Todorovic et al., 2011). Further, PTPN14, a paralogue of PTPN21, interacts with residues in the C-terminus of HPV-16 E7 and particularly residues E80/D81, M84 (Szalmas et al., 2017) and L82, L83 (White et al., 2016) were shown to be important. In order to map the PTPN21 interaction site in the HPV-16 E7 CR3 region, a series of GST pull-down assays was carried out *in vitro* using a panel of HPV-16 E7 mutants to pull-down overexpressed HA-PTPN21 from HEK 293 cells extracts. Figure 42 shows that most of the point mutants assayed are defective in binding when compared with wild type HPV-16 E7, except for the R77A mutant, which seems to bind almost as effectively as GST 16E7 wild type. Residues at T64 and E80/D80 were absolutely required for the interaction, while residues C59, T72 and M84 are also important for the interaction, suggesting that multiple residues in the CR3 region of E7 are important for interaction with PTPN21. These results indicate the interaction between PTPN21 and PTPN14 is not identical, and whilst the carboxy terminal of E7 is required the specific residues involved are different.

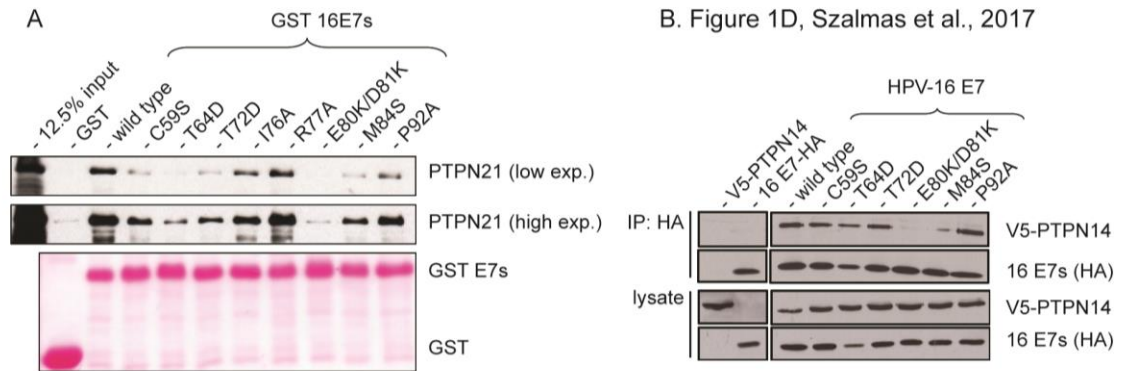


Figure 42. Identification of residues in CR3 region of E7 responsible for binding with PTPN21.

(A). GST 16E7 wild type and CR3 region mutants, as indicated, were incubated at 4°C for 90 minutes with cell extracts from HEK 293 cells overexpressing HA-PTPN21. After extensive washing, bound proteins were analyzed by SDS PAGE and Western blotting. The blot was probed for PTPN21 (upper panel) with anti-HA antibody and levels of GST fusion proteins was ascertained by Ponceau staining of the blot (bottom panel). B. Figure 1D from Szalmas et al., 2017, co-immunoprecipitation of V5-PTPN14 with C-terminal HA tagged HPV-16 E7 wildtype and mutants as indicated. Constructs expressing HPV-16 E7 and PTPN14 as indicated were transfected in HEK293 cells, after 24 hours cells were treated with CBZ for 3 hours and harvested in lysis buffer and immunoprecipitated with anti-HA antibody. Bound V5-PTPN14 was then detected by Western blotting and E7 was detected using anti-HA antibody.

PTPN21 is a degradation target of high-risk E7

Having shown that PTPN21 is a strong interacting partner of HPV E7, we asked if it has a role in HPV E7-induced malignancy. To examine this, a Western blot analysis was done to analyze the steady state levels of PTPN21 in HPV-negative, HEK 293, C33a and HaCaT cells, in HPV-16 positive, CaSki and SiHa cells and in HPV-18 positive HeLa and C4-1 cells. The results are shown in Figure 43A, which shows that the steady-state levels of PTPN21 are lower in HPV-positive cell lines than in HPV-negative cell lines, suggesting that PTPN21 might be downregulated in these cells in the presence of HPV oncoproteins. To determine whether these low levels of PTPN21 in HPV-positive cells are due to proteasome-mediated degradation, HPV-positive HeLa, CaSki and SiHa cells, and HPV-negative C33a cells were treated with proteasome inhibitor Z-Leu-Leu-Leu-al (CBZ, 20μM) for 8 hours. Cells were then harvested and the levels of PTPN21 were then analyzed by SDS-PAGE and Western blotting. The results

shown in Figure 43B indicate that inhibition of the proteasome has no effect on the levels of PTPN21 in HPV-negative C33a cells, while there is an increase in levels of PTPN21 protein in HPV-positive cells after proteasome inhibition. These results suggest that PTPN21 is destabilized in HPV-positive cervical cancer cells via proteasome-mediated degradation.

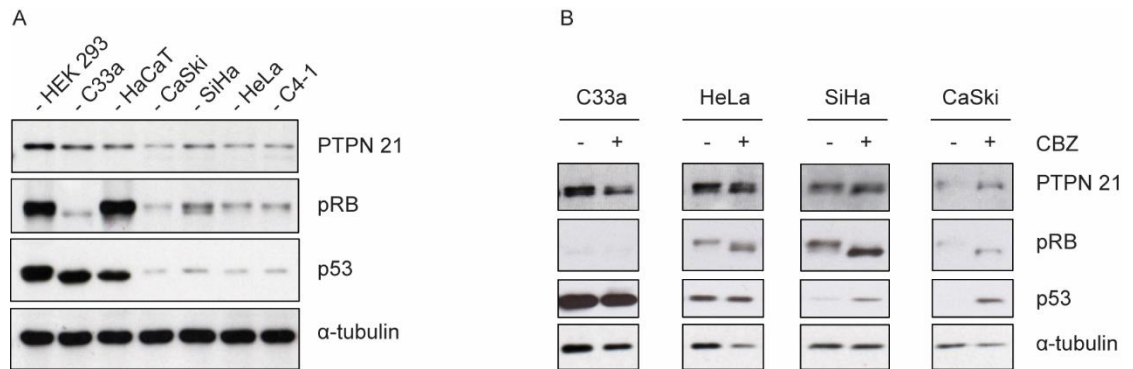


Figure 43. Lower levels of PTPN21 protein in HPV-positive cells are due to its destabilization via proteasome-mediated degradation.

(A). Cell lines, as indicated, were harvested directly in sample buffer and analyzed by SDS-PAGE and Western blot probed for PTPN21, pRB, p53 and α -tubulin. (B). Cell lines, as indicated, were treated with either DMSO or CBZ for 8 hours and then harvested in sample buffer and analysed as before by Western blot probed for PTPN21, pRB, p53 and α -tubulin antibody.

To investigate whether low levels of PTPN21 in HPV-positive cell lines are due to HPV oncoproteins, we transfected HeLa cells with a small interfering RNA (siRNA) against HPV-18 E6/7 or against luciferase as control, siRNA against PTPN21 was used as controls for correct identification of PTPN21. After 72 h cells were harvested and levels of protein expression were assessed by Western blotting. As can be seen from Figure 44A, ablation of E6/E7 expression results in a marked increase in levels of PTPN21. In addition similar results were also obtained in HPV-16 positive SiHa cells (Figure 44B).

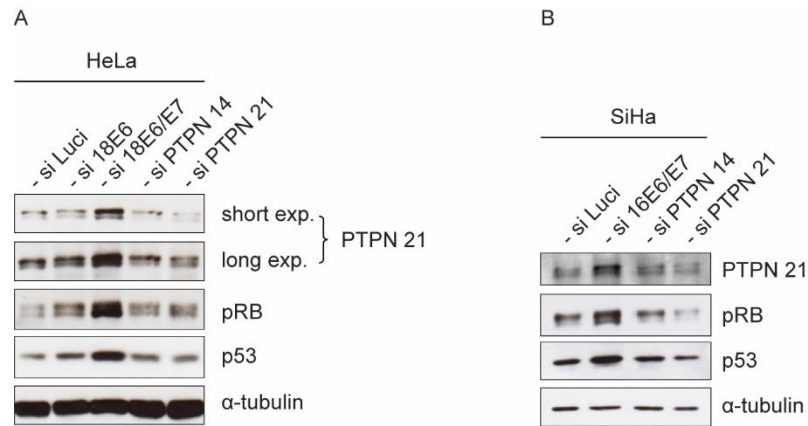


Figure 44. Downregulation of E7 in HPV positive cell lines rescues levels of PTPN21.

Hela (A) and SiHa (B) cells were transfected with the indicated siRNA against luciferase (control), siRNA against E6 or E6/E7, siRNA against PTPN14 or siRNA against PTPN21. After 72 hours, cells were harvested directly in 2X sample buffer and analysed by SDS PAGE and Western blotting for PTPN21, pRB, p53 and α -tubulin.

Since PTPN21 is a potential interacting partner of HPV E7 we further proceeded to investigate whether HPV-16 E7 could target PTPN21 for degradation in a transient-transfection assay. To do this, HEK 293 cells were transfected in duplicate with either HA-tagged PTPN21 alone or in combination with C-terminally FLAG-HA-tagged HPV-16 E7. After 24 hours, one set of the experiment was treated with CBZ for 8 hours and the cells were then harvested and analysed by Western blotting. The results are shown in Figure 45A, where we can see that PTPN21 is targeted for degradation in the presence of E7; and when the proteasome is inhibited using CBZ, the levels of PTPN21 are regained to the levels similar to transient transfection of PTPN21 alone.

Furthermore, as PTPN21 can interact with both high-risk and low-risk E7s, we asked if low-risk E7 is also able to destabilize PTPN21 in transient transfection assay. To do this, we transfected, PTPN21 alone or in combination with C-terminally FLAG-tagged HPV-11 E7, HPV-18 E7 or HPV-16 E7. HPV-16 E7 E80K/D81K was also used in this assay, as it had been shown to have reduced binding with PTPN21 (Figure 42). The cells were then harvested as before and analysed by Western blotting. As can be seen from Figure 45B, both high-risk HPV-16 and HPV-18 E7 are able to degrade PTPN21, although HPV-18 E7 is significantly weaker in this respect and this might correlate with the weaker level

of interaction observed in Figure 41, although more detailed quantification would help clarify this. Similarly, the ability of HPV-16 E7 E80K/D81K to degrade PTPN21 is decreased, suggesting the decrease in ability to bind to PTPN21 might be linked to this activity. In contrast, the low-risk HPV-11 E7 was unable to destabilize PTPN21 despite an apparent strong interaction.

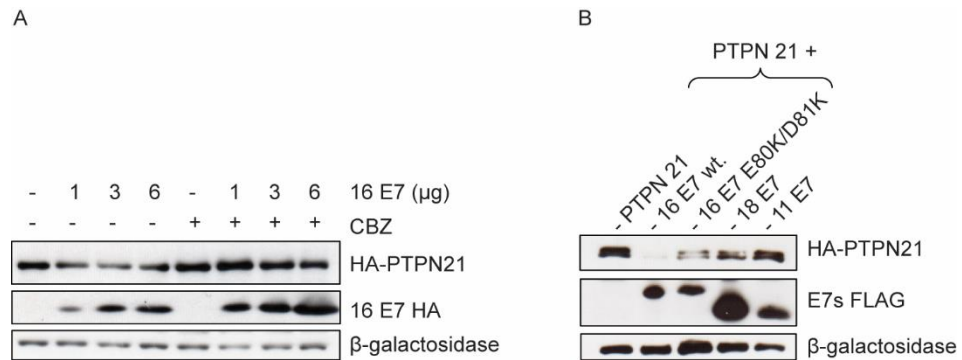


Figure 45. PTPN21 is a degradation target of high-risk HPV E7s.

(A). Plasmid expressing HA-PTPN21 was transfected into 293 cells alone or with increasing amounts of CT-FLAG-HA tagged HPV-16 E7, as indicated, in duplicate. After 24 hours, one set of transfected cells were treated with 20µM of CBZ for 8 hours and cells were harvested directly with 2X sample buffer and analysed by SDS-PAGE and Western blotting for levels of PTPN21 and HPV-16 E7 with anti-HA antibodies. β-galactosidase was included as a control for transfection efficiency. (B). HEK 293 cells were transfected with HA-PTPN21 plus either empty vector or CT FLAG-HA tagged HPV-16 E7 or HPV-16 E7 E80K/D81K or HPV-18E7 or HPV-11E7. β-galactosidase was included as a control for transfection efficiency. After 24 hours cells were harvested directly in 2X sample buffer and analysed with SDS-PAGE and Western blotting for levels of PTPN21 and E7s.

Taken together, these results show that PTPN21 is a binding partner of high-risk and low-risk E7 oncoproteins. The C-terminal CR3 region of E7 is important for binding with PTPN21 and this interaction leads to destabilization of PTPN21 in high-risk HPV E7-expressing cells through proteasome-mediated degradation. Further, the lower levels of PTPN21 found in HPV-positive cells and the subsequent increase in levels of PTPN21 after downregulation of HPV E7 suggests that expression of HPV E7 oncoproteins directly contributes to degradation of PTPN21.

Discussion

Section I

The HPV-18 E7 CKII phospho acceptor site is required for maintaining the transformed phenotype of cervical tumour-derived cells

Despite extensive characterization of the different biochemical functions of the HPV E6 and E7 oncoproteins, and their respective contributions towards the development of cell transformation and malignancy, there is still very little information on the continued requirement for specific activities of E6 and E7 in cells derived from a cervical cancer. This is important, since we know there is an absolute requirement for both oncoproteins for maintaining the transformed phenotype (Butz et al., 2003; Yamato et al., 2008; Yoshinouchi et al., 2003), but which specific biochemical functions of E6 and E7 contribute towards this remains obscure. In this study we have begun to address this question by performing genome editing of the HPV-18 E7 CKII phospho-acceptor site in cervical cancer-derived C4-1 cells. We find that an intact CKII phospho-acceptor site in E7 remains important both for optimal levels of cell proliferation and for maintaining high levels of invasive potential.

Previous studies have shown that the CKII phospho-acceptor site in HPV-16 E7 plays an important role in the HPV life cycle, and in the ability of E7 to bring about cell transformation in a variety of different experimental settings (Chien et al., 2000; Firzlaff et al., 1991). Furthermore, recent studies showed that a variant HPV-16 E7, in which an extra CKII phospho-site was present at N29S, exhibited a marked increase in transforming potential (Zine El Abidine et al., 2017). Many of these activities have been linked to the effects of phosphorylation on the ability of E7 to interact with some of its cellular substrates. Indeed the acidic pocket provided by the CKII consensus motif, and the subsequent increase in negative charge following phosphorylation, have been shown to increase interaction with pocket protein family members (Chemes et al., 2010; Dick and Dyson, 2002; Singh et al., 2005). One consequence of which is the enhancement of HPV-16 E7's ability to overcome pRb-associated cell senescence (P syrri et al., 2004). Phosphorylation of HPV-16 E7 has also been shown to increase interaction with TBP (Massimi et al., 1996). HPV-18 E7 is also widely assumed to be similarly

phosphorylated, however the specific phospho-acceptor site had never been formally verified (Firzlaff et al., 1989). CKII substrate specificity is known to be determined by multiple acidic residues located between -2 and +5 positions relative to the S/T phospho-acceptor site (Marin et al., 1994; Sarno et al., 1996). Based on our mutational analysis we confirmed that S32/S34 are the only CKII phospho-acceptor sites in HPV-18 E7. However, S34 seems to be a more preferred CKII recognition site, although the S32A mutation appears to further enhance phosphorylation of E7 by CKII at S34. The molecular basis for this activity however remains to be determined.

To perform the genome editing of the CKII phospho-acceptor site, we chose C4-1 cells as these cells have only a single integrated copy of HPV-18 DNA (Schneider-Gadicke and Schwarz, 1986). We first confirmed previous studies demonstrating a continued requirement for E6/E7 expression for maintenance of the transformed phenotype in cells derived from a cervical cancer (Butz et al., 2003; Yamato et al., 2008; Yoshinouchi et al., 2003), although this had not formally been demonstrated in C4-1 cells. Following the design of gRNAs for targeting the two serine residues in E7 and subsequent transfection and selection, two mutant cell lines were eventually obtained.

The efficiency of genome editing was low and there are number of possible explanations - the HDR efficiency with which we obtained the number of clones (3 out of 408 screened) lies in the fact that CRISPR/Cas9 HDR genome editing in a given cell line is affected by transfection efficiency as well as cell type and state, the genomic locus and repair template (Saleh-Gohari and Helleday, 2004). For instance, CRISPR/Cas9 and HDR using ssODN, efficiency targeting a given locus in HEK293 cells varies between 20 to 27% while it was 2.2-6% in HUES9 cells (Ran et al., 2013). Further, to target a gene (HPV E6/7 in cervical cancer cells) to which the cells are addicted is yet another challenge, where most of the DSBs caused by gRNAs would lead to these cells entering apoptosis or senescence in absence of a repair compatible to continue cell proliferation. Thus, to enhance the efficiency of editing a HPV oncoprotein sequence in the cervical cancer cells, several techniques including chemical enhancers (e.g. SCR7, inhibitor of DNA ligase IV) (Maruyama et al., 2015), or cell synchronization techniques (Lin et al., 2014), can be used in obtaining a desired genome edit in

future experiments. In addition, off targets of CRISPR/Cas9 is another possibility and various methods for detecting off-targets has been described including deep sequencing (off-target frequency measured range 0.01 to 0.1%) (Cho et al., 2014), web-based prediction tools (Bae et al., 2014), and ChIP-seq (Singh et al., 2015). We used web-based prediction tool, Cas-off finder (<http://www.rgenome.net/cas-offfinder/>, (Bae et al., 2014)), which did not show any off-targets of the gRNAs used in this study based on very stringent off-target prediction tool. Finally, to inhibit repeated targeting of the HPV E7 CKII DNA sequence by the gRNAs used, first a transient transfection method with no selection pressure for retaining the constructs was used and second, the HDR DNA sequence at the putative PAM site was mutated from 'agg' to 'aag' (Figure 18 A) to largely inhibit the potential mutation caused by repeated DSB targeted by gRNA after a successful HDR event (Kwart et al., 2017; Paquet et al., 2016).

Successfully obtaining genome edited clones, demonstrates that mutation of the CKII site per se is not incompatible with continued cell growth and survival. However, upon more detailed analysis, it is clear that mutation of the CKII phospho-acceptor site results in quite marked effects upon the degree of cell transformation. Thus cells with a mutant CKII site in E7 grow more slowly and attain lower saturation densities than wild type E7 cells. This is even more marked when cell growth is assayed in low concentrations of calf serum, where there is a dramatic reduction in the rates of cell proliferation. None of these defects appear related to significant changes in the level of E7 protein expression, as this was unchanged in the mutant lines. It was also unrelated to altered levels of E6 expression, as gauged by the levels of p53. One possible explanation for these slower growth rates under nutrient deprivation could be related to the lower levels of mutant E7 interaction with pocket protein family members, an effect that confirms previous studies showing a role for the acidic patch of the CKII phospho-acceptor site in pocket protein recognition (Genovese et al., 2008; Jones et al., 1997). Indeed, these studies in C4-1 cells are the first to demonstrate unequivocally that an intact CKII phospho-acceptor site in endogenously expressed E7 does influence interaction with pocket proteins. However, whilst this may influence the rates of proliferation, it seems unlikely to be the major cause as changes in pocket protein levels are quite small. It is

tempting to suggest that it is the defect in AKT activation (see below) in the E7 CKII phospho-site mutant lines, especially under low nutrient conditions, which is primarily responsible for the slower growth rates (Pim et al., 2005).

Further analysis of the effects of the CKII mutation upon the transformed properties of the cells revealed marked alterations in the invasive potential of the mutant cell lines. Using matrigel invasion assays, we found that the mutant cells were much less invasive than the parental cells, suggesting that mutation of the E7 CKII phospho-acceptor site reduces the capacity of the cells to invade through a collagen-containing 3D matrix. This defect was not due to a generalized defect in migratory capacity, since the wild type and mutant cells showed no differences in their respective abilities to migrate in a scratch wound healing assay. Instead, this points to a specific defect in migration through a collagen matrix. This therefore led us to examine any potential effects upon MMPs. Using conditioned medium from the different cell lines, we first analysed potential differences in a collagen zymograph assay. To our surprise, we found marked differences between the wild type and mutant cell lines, with the cells harbouring the CKII site mutation exhibiting much lower levels of collagenase activity. This focused our attention on a subset of MMPs, which might be responsible for this defect, and we found that two MMPs, MMP1 and MMP13, were particularly highly secreted by the wild type C4-1 cells, but were largely absent from the conditioned medium of the mutant cell lines. This defect appears to be related to CKII activity, since treatment of the wild type cells with a CKII inhibitor also resulted in a decrease in the amount of secreted MMP1 and MMP13. Similar results were also obtained in HPV-16-positive CaSki cells, where again high levels of MMP1 and MMP13 secretion were dependent upon active CKII, whilst in HPV-negative C33a cells inhibition of CKII had no effect upon MMP secretion. Obviously, confirming the link between E7 phosphorylation and MMP secretion through further genome editing in other HPV-positive cells would be a valuable confirmation, but the technical difficulties in achieving this in cells with multiple HPV copies could render such approaches inconclusive. Finally, overexpression of wild type HPV-18 E7 in the two mutant lines effectively restored the levels of secreted MMP1 to levels similar to those seen in the wild type C4-1 cells, although restoration of MMP13 was much lower. Furthermore,

this rescue of MMP1 secretion was dependent upon an intact CKII phospho-acceptor site in E7, since stable expression of the S32A/S34A double mutant failed to restore the levels of MMP1 secretion. It should be emphasized that when total cell extracts were analysed for the different MMPs, there was very little difference between the different cells, suggesting that the defect was not in expression, but rather in secretion. We also confirmed that MMP13, in particular, also played an essential role in promoting invasion in the matrigel invasion assay, where ablation of expression resulted in a marked decrease in invasive potential, and, where interestingly, the double knockdown of both MMP1 and MMP13 gave the most severe defect in invasive capacity.

A major question at this point was the underlying mechanism. Previous studies have shown that E7 can induce upregulation of diverse MMPs at the transcriptional level (Kaewprag et al., 2013; Srivastava et al., 2017; Wang et al., 2014; Zhai et al., 2005; Zhu et al., 2015), although that did not seem to be the case in the case of the mutant C4-1 cells, where total levels of MMP expression were similar to those seen in the wild type cells. Other studies had shown that E7 could also upregulate AKT activity, which in turn could affect invasive potential (Menges et al., 2006; Srivastava et al., 2017), although the potential role of the CKII phospho-acceptor site of E7 was not investigated in any of these or earlier analyses (Pim et al., 2005). We therefore proceeded to determine whether CKII phosphorylation of E7 might impact upon the levels of AKT activation, and whether this, in turn, might be responsible for the high levels of MMP secretion. Using both transient overexpression systems and the mutant C4-1 cells, it is quite clear that an intact CKII phospho-acceptor site on both HPV-16 and HPV-18 E7 plays an important role in the activation of AKT. Most importantly, however, this activation of the AKT pathway in HPV-positive cells appears to be directly involved in the increase of MMP1 and MMP13 secretion, but not of MMP8 secretion, which is unaffected by either mutation of the E7 CKII phospho-acceptor site or either CKII or AKT inhibition. In contrast, in HPV-negative C33a cells, inhibition of AKT signaling has no effect upon the levels of secreted MMP1 and MMP13. These studies demonstrate that phosphorylation of E7 enhances AKT signaling, which in turn can increase levels of MMP

secretion and invasiveness, and which might also contribute towards increased rates of cell proliferation in low nutrient conditions.

It is intriguing that these studies show a clear difference in MMP1 and MMP13 upregulation, but no change in MMP8. Whilst the molecular basis for this requires further experimentation it is interesting that MMP1 and MMP13 have been found to be upregulated in a variety of different tumours, including cervical, whilst MMP8, on the other hand, has been associated with an anti-invasive effect in certain settings (Akgul et al., 2006; Balbin et al., 2003; Haukioja et al., 2017; Johansson et al., 1999; Korpi et al., 2008; Tian et al., 2018).

It has been demonstrated that AKT pathway is activated in cervical cancer (Menges et al., 2006; Pim et al., 2005) and is one of the main components of epithelial invasion by induction of MMP1 via ETS2-dependent mechanism (Pickard et al., 2012). Activation of AKT pathway leading to MMP secretion and invasion has been described in several models of carcinogenesis (reviewed in (Chin and Toker, 2009)). While it has been demonstrated that HPV E7 activates AKT in earlier studies (Menges et al., 2006; Pim et al., 2005), it was not yet shown that for sustained activation of AKT, phosphorylation of E7 by CKII was required. Then, one of the open questions not yet quite clear is how E7 and/or phosphorylation of E7 contributes to activation of AKT, although it was shown previously that knocking down pRB in keratinocytes was sufficient to increase phosphorylated AKT in raft cultures (Menges et al., 2006), in agreement with regulation of AKT activity through E2F transcription factors during cell cycle through regulation of Gab2 (Chaussepied and Ginsberg, 2004). Gab2 is a scaffold protein that binds to PI3K (phosphatidylinositol 3-kinase) and regulates its activity as well as downstream effectors such as AKT. It is quite tempting to speculate that the increase in levels of pRB in CKII mutant cells might be a response of decreased E2F dependent transcription and decrease in AKT and MMP activation and evaluation of this pathway is of future interest. While this possibility roots from the ability to efficiently engage pRB for interaction and destabilization by HPV E7 upon phosphorylation and E2F dependent transcription, it is also possible that upon phosphorylation HPV E7 might modulate other pathways (for instance, PCP signaling – Vangl1, Discussion

section II) contributing to cancers phenotypes including proliferation, invasion and migration.

In summary, these studies demonstrate that the activity of the E7 CKII phospho-acceptor site remains functional and relevant in cells derived from cervical cancers, and indicate that the CKII phospho-acceptor site remains a very attractive candidate target for therapeutic intervention in HPV-induced malignancy.

Section II

Planar cell polarity protein Vangl1 is targeted by Human Papillomavirus E7 oncoprotein

Previous studies have shown that phospho-specific interactions of HPV E7 with cellular proteins in cervical cancer derived cells have diverse implications including cellular transformation (Barbosa et al., 1990; Firzlaff et al., 1991; Heck et al., 1992), cellular growth and differentiation (Muller et al., 1999; Psyrri et al., 2004), cell cycle regulation (Chien et al., 2000), and actin polymerization (Rey et al., 2000). Intriguing results from genome editing of the phospho-acceptor site in HPV-18 E7 positive C4-1 cells led us to design a proteomic screen specifically aimed at identifying novel targets of HPV-E7 that are dependent on its CKII phosphorylation. First, we used phosphorylated- and non-phosphorylated E7 peptides as bait in a pulldown assay from HaCaT cells, and by mass spectroscopic analysis we identified Vangl1 as a phospho-specific interactor of HPV-16 E7. Second, we verified this interaction *in vivo* in cells using immunoprecipitation assays. Third, we showed that Vangl1 co-operates with HPV-16 E7 in cell transformation, suggesting that CKII phosphorylation of HPV E7 and interaction with Vangl1 could be an important therapeutic target in HPV-induced cancers.

By using a proteomic analysis of the cellular proteins binding to a phosphorylated peptide corresponding to the CR2 region of E7, we identified Vangl1, a planar cell polarity protein, as a phospho-specific interactor of E7. For this analysis we used a variant peptide with additional phospho-acceptor site at N29S, previously described to have enhanced transforming properties due to acquisition of this phospho-acceptor site (Zine El Abidine et al., 2017). Further, we verified this target using western blot from the peptide pull-down and could show that this interaction is specific to phosphorylated peptide of E7, but is undetectable in non-phosphorylated or phosphomimic peptide of E7, suggesting that only phosphorylation at the CKII site of E7 is highly important for this interaction, and an amino acid phosphomimic does not function in this context. We could also pull-down higher levels of pRB with phosphorylated E7 peptide than with non-phosphorylated peptide, in line with previous studies showing that the negative

charge acquired by phosphorylation increases E7 interaction with pocket protein family members (Chemes et al., 2010; Dick and Dyson, 2002; Singh et al., 2005). Further, we verified the interaction *in vivo* in cells using HEK 293 cells by overexpressing FLAG-Vangl1 and C-terminus HA-FLAG-tagged HPV-16 E7 wildtype, N29S and S31A/S32A mutants. Using co-immunoprecipitation, we could show that both the full-length HPV-16 E7 and the N29S variant can interact with Vangl1, consistent with results from the peptide pulldown, while the S31A/S32A mutant shows only a weak interaction. Further, inhibition of phosphorylation of E7 using a specific CKII inhibitor CX4945 showed that this interaction requires CKII activity, which further supports a role for CKII phosphorylation of E7 being required for the interaction and that the defect in interactions is not simply a reflection of introducing mutations at the two serine residues. The CKII phospho-acceptor site is conserved between high-and low risk E7s. Indeed, earlier studies on E7 phosphorylation has shown that high-risk and low-risk E7 could be phosphorylated on equivalent residues in the CR2 region albeit phosphorylation of low-risk E7 is weaker in kinetics compared to high-risk E7s (Barbosa et al., 1990; Firzlaff et al., 1989). Using co-immunoprecipitations assays together with CKII inhibition in overexpression assays, we showed that this interaction is conserved across high-risk HPV-16, HPV-18, and HPV-31 and also with the low-risk HPV-11 E7s, although interaction with HPV-18 E7 appeared much weaker and probably reflects a weaker level of association with Vangl1. Nonetheless, co-immunoprecipitation assays with HPV-18 E7 in C4-1 wildtype and CKII genome edited mutant cells showed that interaction of Vangl1 with endogenous HPV-18 E7 was much higher in wildtype cells. It was interesting to note that levels of Vangl1 were lower in the CKII mutant cells compared with wildtype cells, correlating with the invasive ability of these cells. Studies in different cancers, including breast (Anastas et al., 2012; Luga et al., 2012), colon (Lee et al., 2004), head and neck (Lee et al., 2009), glioblastoma (Wald et al., 2017), and hepatocellular carcinoma (Cetin et al., 2015; Yagyu et al., 2002) have shown that high levels of Vangl1 is positively correlated with migratory and invasive abilities. In agreement with this, using a BRK cell transformation assay, we could show that Vangl1 enhances cellular transformation and can cooperate with E7 in these assays, supporting previous studies of its implication in various cancers.

Future studies will be focused towards further characterizing the role of the Vangl1 and E7 interaction, and its modulation in planar cell polarity. HPV E6 and E7 have been shown to perturb several members of cell polarity including, Dlg (Gardioli et al., 1999), Scribble (Nakagawa and Huibregtse, 2000), Magi (Glaunsinger et al., 2000), and Par3 (Rh E7) (Tomaic et al., 2009), either by proteasomal degradation leading to disruption of the stoichiometric equilibrium of these complexes or by re-localization of the protein. Thus our studies are particularly intriguing, in that this now also links E7 with the regulation of cell polarity, and indicates that high risk HPVs have mechanisms for modulating cell polarity in a wide variety of pathways. While the distribution and cellular localization of Vangl1, as a core component of PCP, plays a major role in epithelial cell polarity how E7 is affecting this still remain to be determined. However, our preliminary studies indicate that E7 may be contributing towards the increased levels of expression of Vangl1 which would be consistent with an increase in MMP expression and increased invasive potential. Whether Vangl1 is directly affecting MMP secretion, or whether it is modulating AKT which in turn modulates MMPs are all still major open questions.

One clue to Vangl1 being involved in activation of AKT comes from Vangl1 associated another PCP component, Prickle1, which was recently shown to interact with RICTOR, a member of the mTOR2 complex (Daulat et al., 2016). In this study, the integrity of Prickle1-MINK1-RICTOR complex was shown to be required for activation of AKT, regulation of focal adhesions, and breast cancer cell migration. Further, upregulation of Prickle1 in basal breast cancers was characterized by high metastatic potential and associated with poor metastasis-free survival (Daulat et al., 2016; Daulat and Borg, 2016). It may well be that Vangl1 associated Prickle1 is involved in activation of AKT via mTOR2 and phosphorylation of E7 and Vangl1 interaction might be potentiating activation of AKT via Prickle1-mTOR2 complex, which needs further investigation. However, the fact that Vangl1 appears to cooperate with E7 in a cell transformation assay, and also interacts with multiple HPV E7 oncoproteins in a CKII dependent manner provide strong justification for an important role for Vangl1 both in HPV induced malignancy and in the HPV life cycle.

Section III

PTPN21 is a degradation target of high-risk HPV E7

Previous studies have indicated that both PTPN14 and PTPN21 are potential binding partners of HPV E7s (Rozenblatt-Rosen et al., 2012; White et al., 2012), however, the PTPN21 and E7 interaction has not been fully characterised. In this work, we have begun to address the potential role of this interaction. First, we verified the interaction between PTPN21 and HPV-16, HPV-18 and HPV-11 E7s. The interaction of PTPN21 is mediated by the C-terminal region of E7, similar to the PTPN14 interaction region in HPV-16 E7, although the specific amino acid residues involved are different, possibly due to specific changes in interaction residues in PTPN14 and PTPN21 although, these phosphatase share high sequence similarity. The E80/D81 and T64 amino-acid residues in HPV-16 E7 were particularly found to be responsible for interaction with PTPN21. Then, using HPV-positive and HPV-negative cell lines we could show that steady state levels of PTPN21 are lower in HPV-positive CaSki, HeLa, SiHa and C4-1 lines, indicative of destabilization of PTPN21 by proteasomal degradation. Further, using proteasome inhibition, the levels of PTPN21 in HeLa, SiHa and CaSki cells could be rescued, however, the levels of PTPN21 were unaffected in HPV-negative C33a cells. Finally, we could show that downregulation of E6/E7 expression using siRNA in HeLa and CaSki cells, could rescue levels of PTPN21, suggesting that PTPN21 interaction with E7 leads to its degradation. We corroborated these results with overexpression studies, where we could show that PTPN21 destabilization by HPV-16 E7 is mediated by the proteasome, while low-risk HPV-11 E7 and the PTPN21-binding-defective mutant of HPV-16 E7, E80K/D81K, was unable to destabilize PTPN21 to the similar levels as wildtype high-risk E7s. These studies indicate that, as with PTPN14, PTPN21 also interacts via the C-terminus of HPV E7, and high-risk E7s destabilize it via the proteasome.

Previous studies identified that the PTP domain of PTPN14 was responsible for the interaction with HPV-16 E7 (White et al., 2016). Recent structural studies (Yun et al., 2019) have identified the key residues in the PTPN14 PTP domain and in HPV-18 E7 that are responsible for this interaction. The intermolecular

hydrophobic interactions between these two proteins are mediated by the F90 and L91 (consensus L83 and M84 in 16 E7), and M61 and L62 (consensus I54 and V55 in 16 E7) residues in HPV-18 E7 and the K1043, F1044, G1055, W1070 and L1026 residues of the PTPN14 PTP domain. Additionally, the HPV-18 E7 R84 residue (conserved R77 in HPV-16 E7) was shown to bind with electrostatic interactions to the E1095 of the PTPN14 PTP domain. These findings are in broad agreement with consensus residues of interaction in HPV-16 E7 identified by recent studies, particularly the residues L83 and M84 (Szalmas et al., 2017; White et al., 2016). Further, the PTP domains of PTPN14 and PTPN21 are highly conserved, particularly residues in the hydrophobic regions of the phosphatase domain in PTPN14 (L1026, F1044, G1055 and W1070) were identical in PTPN21, suggesting a similar potential interaction; and indeed this was found to be the case, where it was shown that the HPV-18 E7 C-terminus interacts with PTPN21 with a dissociation constant of 5.81 nM using isothermal titration calorimetry (Yun et al., 2019), in good agreement with the interaction we observed between C-terminus of HPV-16 E7 and PTPN21, although the specific residues are not the same, most possibly due to obvious difference between specific residues in CR3 regions of HPV-16 and -18 E7s contributing to changes in steric interaction clouds.

As with PTPN14, we show that both high-risk and low-risk E7s bind to PTPN21, however, only high-risk E7s are able to destabilize PTPN21, suggesting a function of this interaction conserved across several HPV types independently of the ability to degrade it via proteasome. A recent study has shown that high-risk E7 potentially destabilize PTPN14 to limit keratinocyte differentiation contributing to carcinogenesis (Hatterschide et al., 2019). Further HPV-16 E7 E10K mutant and the low-risk HPV-6 E7, unable to destabilize but effectively bind PTPN14 was shown to inhibit keratinocyte differentiation to similar levels as high-risk E7s, however, how the interaction with PTPN14 could impact keratinocyte differentiation was not shown (Hatterschide et al., 2019). Thus, it is very appealing to speculate in terms of both PTPN14 and PTPN21 interaction with HPV E7s has a conserved function in HPV life cycle, yet to be uncovered.

Further we observed a weaker interaction of HPV-18 E7 compared to -16 E7 with PTPN21, however, transient overexpression assay suggests, both are efficient in destabilizing the levels of PTPN21 and is further supported by increase in levels of PTPN21 upon downregulation of E6/E7 in HeLa and SiHa cells. Furthermore, the p600/UBR4 ubiquitin ligase has been shown to be responsible for proteasome-mediated destabilization of PTPN14 (Szalmas et al., 2017; White et al., 2016). The ligase involved in degradation of PTPN21 is currently under investigation and it is likely that the UBR4/p600 might be involved in destabilization of PTPN21, while additional ligases; mainly Cul1 (Oh et al., 2004), Cul2 (Huh et al., 2007; Kamio et al., 2004; White et al., 2012) and Cul3 (White et al., 2012) that are known to associate with HPV E7, also needs be screened. More recently it was shown in case of hematopoietic stem cells (HSCs) that depletion of PTPN21 enhances cell stiffness and increases mobility in HSCs in part by phosphorylation of cytoskeletal protein, Septin1 at Tyr246, a substrate of PTPN21 (Ni et al., 2019). Whether destabilization of PTPN21 by high-risk E7 affect Septin1 modulating cytoskeletal architecture needs further investigation.

Our previous studies using BRK transformation assays suggested that for efficient abrogation of HPV-16 E7's transforming activity both PTPN14's substrate recognition function and enzymatic activity was required (Szalmas et al., 2017), suggesting modulation of substrates and enzymatic activity of PTPN14. Until now, several substrates including beta-catenin (Wadham et al., 2003), YAP (Liu et al., 2013), p130Cas (Zhang et al., 2013), RIN1 (Ras and Rab interactor 1) and PRKCD (protein kinase C-d) (Belle et al., 2015) have been described for PTPN14, however, how these substrates are modulated upon E7 interaction with PTPN14 is yet to be determined. While both PTPN14 and PTPN21 have been demonstrated to bind with YAP and negatively regulate its oncogenic activity (Li et al., 2016; Liu et al., 2013), it is possible that both of these phosphatases might function to compensate each other's function in the Hippo pathway. However, whether E7 mediated downregulation of PTPN14/21 plays a role in activation of Hippo pathway is not clear and is of future interest. Furthermore, PTPN21 has been implicated in several signaling pathways: modulating STAT3 signaling (Jui et al., 2000), vesicle trafficking (Dorner et al.,

1998; Siddiqui et al., 2019), SRC dependent EGF signaling (Cardone et al., 2004), cellular migration and adhesion (Carlucci et al., 2008). Future studies will be aimed at determining which of these, or other, pathways are modulated as a result of targeting PTPN21 by HPV E7s.

In summary in this thesis I have shown intimate interactions between HPV E7 oncoproteins and post translational modification machinery. This includes association with tyrosine phosphatases as one means by which E7 can modulate the post translational modification of some of its potential target proteins. But more importantly, also a very strict regulation of E7 function through post translational modification, and in particular through the CKII phosphor-acceptor site. This has a profound effect on the ability of cervical cancer derived cell lines to proliferate, reach high saturation density and invade, all of which indicates that targeting E7 phosphorylation might offer important therapeutic potential. In addition, molecularly this CKII phospho modification links E7 phosphorylation to increased levels of AKT activation, interaction and stabilisation of Vangl1 and stimulation of MMP secretion and an acquisition of an invasive phenotype. Whether these particular activities of E7 are linked, or represent separate biological and biochemical activities remains to be fully resolved.

Materials and Methods

Cells

HEK 293, C33a, HaCaT, CaSki, SiHa, HeLa and C4-1 cell lines were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, glutamine (300 µg/ml), and penicillin-streptomycin (100 U/ml). All cell lines were cultured in an incubator at 37°C with 10% CO₂.

Inhibitors and chemical reagents

The inhibitors and chemicals used for the experiments are as follows: 2.5 µM (for C4-1 cells) or 50 µM (for HEK 293 cells in overexpression experiments) Silmitasertib (CX-4945), 5µM AKT inhibitor (124005-Calbiochem), 15µg/mL PI3K inhibitor (LY294002) and 10µg/mL Mitomycin C, 20 µM MG132 or CBZ (Z-L-Leu-D-Leu-L-Leu-al), 2.5mM thymidine (Sigma Aldrich).

Plasmid constructs

The GST 18 E7 S32A/S34A was synthesized by the Gene Art Gene Synthesis protocol (Invitrogen) and cloned into the BamHI/EcoRI sites of the pGEX2T for GST fusion protein expression. The wildtype pGEX:18E7 plasmid was a kind gift from Karl Mürger. The same plasmid was used to generate pGEX:18 E7S32A and pGEX:18E7S34A, using a modification of the QuickChange site-directed mutagenesis system (Stratagene), according to the manufacturer's instructions, with the following primers: S32A forward primer 5'-TGT CAC GAG CAA TTA GCG GAC TCA GAG GAA GAA-3' and S32A reverse primer 5'- TTC TTC CTC TGA GTC CGC TAA TTG CTC GTG ACA-3'; S34A forward primer 5'-GAG CAA TTA AGC GAC GCA GAG GAA GAA AAC GAT-3' and S34A reverse primer 5'-ATC GTT TTC TTC CTC TGC GTC GCT TAA TTG CTC-3'. pSpCas9(BB)-2A-GFP (PX458) was purchased from Addgene.

The C-terminal FLAG-tagged HPV-18 E7 wild type and S32A/S34A CMV plasmid constructs were generated by amplification and sub-cloning of the E7 coding sequences from the wild-type pGEX:18E7 and pGEX:18 E7S32A/S34A

construct as templates respectively into the pCMV Neo Bam (XhoI) empty vector (kindly provided by J. Mymryk). The primers used were the following: HPV-18 E7 forward primer 5'-CGA CGG ATC CGA TTC GAG ACC ATG CAT GGA CCT-3' and reverse primer 5'-GCA TCT CGA GCT ACT TGT CAT CGT CGT CCT TGT AGT CCT GCT GGG ATG C-3'. The amplified sequences were visualized by agarose gel electrophoresis, purified by using a QIAquick gel extraction kit (Qiagen), digested with BamHI and XhoI restriction enzymes, and ligated into pCMV Neo Bam (XhoI).

The C-terminal FLAG/HA-tagged pCMV HPV-16 E7 plasmid was a kind gift from Karl Munger (Gonzalez et al., 2001). The same plasmid was used to generate pCMV HPV-16 E7 S31A/S32A using the forward primer 5'-CTC TAC TGT TAT GAG CAA TTA AAT GAC GCC GCA GAG GAG GA-3' and the reverse primer 5'-TCA TCC TCC TCC TCT GCG GCG TCA TTT AAT TGC TCA TAA CA-3' using the QuickChange site-directed mutagenesis system (Stratagene) as described previously. The pEGFP 18 E7 was generated by PCR amplification and sub-cloning of E7 coding sequences from wildtype pCMV:18E7-FLAG plasmid as template, using the forward primer 5'-ATG CGA ATT CAT GCA TGG A-3' and the reverse primer 5'-GCA TTC TAG ATT ACT GCT G-3' into EcoRI and XbaI restriction sites of the pCANmyc-EGFP 16E7 (JMB-04093) plasmid (kindly provided by J. Mymryk). pEGFP-C1 empty plasmid was from Invitrogen. The HA-PKB expression plasmid has been described previously (Pim et al., 2005). pcDNA:FLAG-Vangl1 was kindly provided by Pablo Rodriguez-Viciana (Young et al., 2013). The pcDNA3:V5-PTPN14 plasmid was kindly provided by Jianmin Zhang (Liu et al., 2013).

Production and purification of GST-fusion proteins

The appropriate expression plasmids were transformed into *E. coli* strain DH5- α . The clones harboring plasmids were grown in 40ml of Luria Broth (LB) culture medium containing 75 μ g/ml Ampicillin (Sigma) overnight at 37°C. The overnight grown cultures were then transferred into 400ml of LB containing 75 μ g/ml Ampicillin and incubated at 37°C for 1h. The isopropyl- β -D-thiogalactopyranoside (IPTG) (Sigma) to a final concentration of 1 mM was added to induce recombinant protein expression and the culture was incubated for approximately

3h at 37°C on a shaker. Post IPTG treatment, the bacteria were harvested by centrifugation at 5000 rpm for 5 minutes. Supernatants were discarded and the bacterial pellets were lysed with 5-10ml of 1X PBS containing 1% Triton X- 100 and sonicated twice for 30 seconds at 80% amplitude. The lysates were then centrifuged again at 10,000 rpm for 15 minutes. The supernatants were collected and incubated with glutathione-conjugated agarose beads on a rotating wheel overnight at 4°C. The GST-fusion protein containing beads were then centrifuged at 2000 rpm for 1 minute and the supernatant was discarded. The beads were washed thrice with 1X PBS containing 1% Triton X-100. The GST-fusion protein-bound containing beads were then stored with 20% glycerol at -20°C.

***In vitro* translation**

Proteins were translated in vitro using a TNT kit (Promega) and radiolabeled with [35S] methionine (Perkin Elmer). The purified GST-fusion proteins were then incubated with in vitro-translated proteins for 1hr at 4°C. Proteins were washed with 1X PBS containing 0.1% NP-40 and were analyzed by SDS-PAGE and autoradiography.

***In vitro* phosphorylation assay**

Purified GST fusion proteins were incubated with CKII enzyme (NEB) in 20 µl kinase buffer (20 mM Tris-HCl [pH 7.5], 5 mM MnCl₂) in the presence of 50 µCi [γ-32P] ATP (2,000 Ci/mmol) for 15 min at 30°C. After extensive washing with kinase wash buffer (20 mM Tris-HCl [pH 7.5], 5 mM MnCl₂, 0.1% NP-40), GST fusion proteins were subjected to SDS-PAGE and autoradiographic analysis (Massimi et al., 1996).

DNA and siRNA transfections

HEK 293 cells were transfected using the calcium phosphate precipitation method (Wigler et al., 1979).

For transfection of siRNAs, C4-1 cells were seeded at 2 x 10⁵ cells per 60 mm petri plate and grown overnight in a humidified CO₂ incubator. Lipofectamine RNAiMAX (Invitrogen) was used to transfect siRNAs against luciferase, HPV-18

E6 (5'-CUC UGU GUA UGG AGA CAC AT-3'), HPV-18 E6/E7 (5'-CAU UUA CCA GCC CGA CGA G-3'), MMP1 (Dharmacon), MMP13 (Dharmacon), PTPN14, PTPN21 according to the manufacturer's instructions.

Design of gRNAs, homology directed repair (HDR) template and screening strategy of genome edited clones

A couple of gRNAs targeting CKII phosphorylation site in HPV 18 E7 genomic region was designed using online gRNA design platform – CRISPR MultiTargeter (<http://www.multicrispr.net/>). gRNA oligos (gRNA1 – 5'-CAC CGC GAG CAA TTA AGC GAC TCA G-3' and 5'-AAA CCT GAG TCG CTT AAT TGC TCG C-3'; gRNA2 – 5'-CAC CGT TAA TTG CTC GTG ACA TAG A-3' and 5'-AAA CTC TAT GTC ACG AGC AAT TAA C-3') were then annealed and cloned into the BbsI restriction site in pSpCas9(BB)-2A-GFP (PX458).

Single stranded oligonucleotide (ssODN) as donor template for homology-directed repair (HDR) was designed as 100bp homology arm flanking the predicted double strand break site to abolish phosphorylation, substituting serine 32 with alanine (agc to gcc) and serine 34 with alanine (tca to gca). The designed mutagenesis also included a unique Hga I restriction site, to allow screening of the edited clones by genomic DNA isolation, PCR amplification of edited region and Hga I restriction digestion.

gRNA transfection, FACS sorting and surveyor assay

C4-1 cells were transfected with pSpCas9(BB)-2A-GFP or with the same construct with gRNA1 or gRNA2 using the Amaxa Cell Line Nucleofector Kit C according to the manufacturer's instructions. Transfected cells were incubated for 72 hrs in a humidified CO₂ incubator and then harvested with trypsin, washed once with PBS and resuspended in 5mM EDTA [pH 8.0] in PBS for FACS (BD FACS Aria II) sorting. GFP-positive cells were collected and genomic DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega) according to the manufacturer's instructions. The HPV 18 E7 ORF was amplified (414 bp) using forward primer 5'-CCA ACG ACG CAG AGA AAC AC-3' and reverse primer 5'-AAA CCA GCC GTT ACA ACC CG-3'. Amplicons were then denatured and reannealed as follows: 95°C for 10 min, 95°C to 85°C for 1 min, 85°C to

75°C for 1 min and so on for every 10°C decrease in temperature for 1 min and finally a hold at 25°C for 1 min using a thermal cycler to allow DNA heteroduplex formation. The heteroduplexes were then treated with Surveyor nuclease and Surveyor enhancer (IDT) and analyzed on a 2% agarose gel. The cleavage intensity of the gRNAs was calculated by analyzing band intensities using the ImageJ program. The indel percentage caused by respective gRNA was then calculated as described by Ran et al. (Ran et al., 2013).

Isolation, screening and verification of C4-1 mutant lines

C4-1 cell lines were transfected as described above with pSpCas9(BB)-2A-GFP gRNA1, together with the ssODN HDR template and incubated in a humidified CO₂ incubator. Seventy-two hours after transfection, the top 10% GFP-positive cells were sorted, as described above, and seeded in limiting dilution in a 100 mm diameter petri dishes for isolation of single cell clones using cloning chambers, or in 96 well tissue culture plates. Genomic DNA extraction of the single cell colonies and PCR amplification of the E7 ORF was performed as before. Then, individual PCR amplicons were restriction digested with Hga I restriction enzyme and analyzed on a 2% agarose gel. Clones positive for Hga I restriction digestion were further verified by Sanger sequencing for genome editing at the CK II site.

Determination of growth kinetics

C4-1 wild type and mutant lines were seeded at 3×10^5 cells in a 60 mm petri dishes in DMEM with 10% fetal bovine serum. Cells were harvested with trypsin, dispersed and counted using a hemocytometer every single day or two. For determination of growth in low serum, the experiment was performed independently in DMEM with 1% and 0.2% fetal bovine serum. Cell counts were analyzed using Graphpad Prism 7.0 to generate a growth curve.

Antibodies, Western blotting and co-immunoprecipitation

The following antibodies were used for western blotting. Mouse monoclonal anti- β -galactosidase antibody from Promega, mouse monoclonal anti-V5 antibody from LifeTechnologies, rabbit polyclonal anti-PTPD1/PTPN21 (ab12550)

antibody from Abcam, mouse monoclonal anti-MMP14 (MAB3328) antibody from Millipore, mouse monoclonal anti-p53 antibody (DO-1), rabbit polyclonal anti-p130 antibody (C-20), mouse monoclonal anti-HPV18 E7 antibody (F-7), mouse monoclonal anti-HPV-16 E7 (NM2) antibody, mouse monoclonal anti-MMP-1 antibody (SB12e), mouse monoclonal anti-MMP-8 (B-1) antibody, mouse monoclonal anti-MMP13 (C-3) antibody, mouse monoclonal anti- α -actinin antibody (H-2), mouse monoclonal anti-GFP Antibody (B-2), rabbit polyclonal Cyclin A antibody (H-432) from Santa Cruz Biotechnology; mouse monoclonal anti-Rb (G3-245) antibody from BD Pharmingen, mouse monoclonal anti- α -tubulin antibody, mouse monoclonal Anti-VANGL1 (clone CL0241) antibody, mouse monoclonal anti-HA-peroxidase (clone HA-7) antibody, mouse monoclonal anti-FLAG-M2-peroxidase from Sigma, mouse monoclonal anti-18E6 antibody (N-terminus #399; Arbor Vita Corporation), rabbit Akt antibody #9272, rabbit anti phospho-Akt (Ser473) antibody #9271 from Cell Signaling. HPV-16 E7 pS31/S32 peptide antibodies was generated by Eurogentec (detail procedure in Appendix I) (peptide sequence: C+EQLND-S(PO₃H₂)S(PO₃H₂)-EEED and validated by ELISA and Western blotting. Secondary anti-rabbit HRP and anti-mouse HRP antibodies were obtained from Dako.

For Western blotting, total cell extracts were obtained by lysing the cells directly in 2X SDS-PAGE sample buffer and were then separated by SDS-PAGE and blotted on 0.22- μ m nitrocellulose membrane. Membranes were blocked in 5% non-fat dry milk in TBST (20mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) for 1 h and probed with appropriate primary and secondary antibodies. The blots were then developed using the ECL Western blotting detection reagent (GE Healthcare) according to the manufacturer's instructions.

For detection of extracellular MMPs, confluent cells were cultured in serum-free medium for 48 hours and conditioned media were harvested, concentrated 10 times using Amicon Ultra-4 centrifugal devices and equal amounts of proteins were mixed with 2X SDS-PAGE sample buffer and processed as described previously for Western blotting.

For co-immunoprecipitation, wild type and CKII phospho-acceptor site mutant C4-1 cells were harvested using lysis buffer (50mM HEPES pH7.4, 150mM NaCl,

1mM MgCl₂, 1mM NaF, 1% Triton-x-100, protease inhibitor cocktail I [Calbiochem]) and incubated with 1µg of mouse monoclonal HPV-18 E7 antibody overnight at 4°C. Mouse monoclonal IgG antibody against GFP was used as a control. After incubation, immune complexes were incubated further with Protein A beads (Immobilized protein A 300, Repligen) for 90 minutes at 4°C, followed by four washes in the lysis buffer. Immuno-precipitates were then run on SDS PAGE and analysed by Western blotting.

Validation of HPV-16 E7 phospho-specific antibody

Purified GST fusion proteins of HPV-16 E7 were incubated either with or without CKII enzyme (NEB) in kinase buffer (25mM Tris pH7.5, 70mM NaCl 10mM MgCl₂) in the presence of ATP for 15 min at 30°C. After extensive washing with kinase wash buffer (kinase buffer with 0.1% NP-40), GST fusion proteins were separated by SDS PAGE and transferred to nitrocellulose membrane and stained with Ponceau for levels of GST fusion proteins. The blots were then probed for HPV-16 E7 phospho-specific antibody.

Matrigel Invasion Assays

Matrigel Invasion Assays were performed as described previously (Massimi et al., 2012). Briefly, Matrigel invasion chambers (Corning BioCoat Matrigel Invasion Chamber) were brought to room temperature and rehydrated with DMEM without serum for 2 hours in a humidified CO₂ incubator. Wild type and mutant C4-1 cells were seeded at 1×10⁵ cells in 200 µl of growth medium into the upper chamber. After allowing the cells to attach for 1 h, the medium in the upper chamber was replaced with DMEM without serum, while DMEM with 2% serum was added to the lower chamber as chemoattractant. After 22 hours, DMEM and any cells remaining in the upper chamber were removed by wiping with a cotton swab. Cells that had invaded the lower chamber were then fixed and stained with 0.5% Crystal Violet in 5% glutaraldehyde for 10 mins. After washing the excess stain with distilled water, the membrane was removed from the insert housing and placed on a microscope slide for imaging and analysis using a transmitted light microscope at 20X magnification. At least three fields

per membrane were counted for each cell line. Invaded cell counts were analyzed using Graphpad Prism 7.0.

Wound healing assay

Confluent wild type and CKII mutant C4-1 cells were scratched with a sterile Artline p2 pipette tip. The cells were then washed twice with PBS and photographed immediately and after 24 hours. The decrease in area of the scratch was analysed and quantified using the Image J and Prism programs.

Gelatin Zymography

Wild type and mutant C4-1 cell lines were incubated in a serum-free medium for 48 hours and conditioned media from the cell lines were concentrated 10 times using Amicon Ultra-4 centrifugal devices. Equal amounts of proteins were mixed with non-reducing sample buffer (125mM Tris-HCl pH 6.8, 4% SDS, 20% glycerol and 0.01% bromophenol blue) and run on a 10% SDS-PAGE containing 0.1% gelatin. The gel was renatured by washing with 2.5% Triton X-100 in 50mM Tris pH 7.4, 5mM CaCl₂ and 1 μ M ZnCl₂ for 1 hr. After rinsing briefly with deionized water, the gel was incubated overnight at 37 °C in 1% Triton X-100, 50mM Tris pH 7.4, 5mM CaCl₂ and 1 μ M ZnCl₂ and stained with Coomassie staining solution (0.5% Coomassie G250, 40% Methanol, 10% Acetic acid and 50% deionized water) for 1 hr and de-stained in 40% Methanol, 10% Acetic acid and 50% deionized water, until clear bands of hydrolyzed substrate were visualized.

Preparation of HaCaT cell lysate, peptide pull down and Mass spectroscopy

Soluble proteins were extracted from 80% confluent HaCaT cells by incubation for 10 min on ice in lysis buffer (50mM HEPES pH7.4, 150mM NaCl, 1mM MgCl₂, 1% Triton-x-100, protease inhibitor cocktail I [Calbiochem]). The cell lysate was collected by scraping the plate into a pre-chilled eppendorf tubes and by centrifugation at 14000 rpm in a benchtop centrifuge for 10 min at 4°C.

500 μ g of each peptide, in lysis buffer, was bound to streptavidin-conjugated magnetic sepharose beads (Streptavidin-MagSepharose, GE Healthcare) by

incubation at 4°C on a rotating wheel for 1h, then washed three times with lysis buffer. The cell extract was pre-cleared by incubation with empty streptavidin conjugated magnetic beads at 4°C on a rotating wheel for 1h. After removal of the pre-clearing beads, the extract was incubated at 4°C on a rotating wheel for a further 2h with each of the biotinylated peptides bound to streptavidin-conjugated magnetic sepharose beads. The beads were washed three times with lysis buffer without protease inhibitors, transferred to fresh eppendorf tubes and washed twice more with lysis buffer without either protease inhibitors or Triton-x-100. 5% of the beads were taken for western blot analysis and the remainder were subjected to trypsin-digest and the products analysed by mass spectroscopy, as described previously (Tomaic et al., 2009).

Primary baby rat kidney (BRK) cell transformation assay

Primary epithelial cells were obtained from the kidneys of 9-day-old Wistar rats as described previously (Matlashewski et al., 1987). After 24 h, the cells were transfected by calcium phosphate precipitation (Wigler et al., 1979) with plasmids expressing EJ-ras or with HPV-16 E7 or with plasmids expressing Vangl1. The cells were maintained under G418 selection for 2 weeks and then fixed and stained with Giemsa. The stained colonies were counted, and a t-test was used to compare mean number of colonies formed in different set of transfections as described in statistical methods.

Statistical Methods

All experiments were performed at least thrice, and data are shown as mean and standard error of mean. Statistical significance was calculated using the GraphPad prism software. To compare two groups the paired Student's t-test was performed. A p-value below 0.05 was considered statistically significant and throughout the p-values have been defined as follows *p < 0.05, **p < 0.005, ***p < 0.0005, while "ns" represents a non-significant p-value above 0.05. For the quantification of protein levels from western bolts, the films were scanned, and the intensity of bands was measured using ImageJ software. The final relative quantification values are the ratio of net band to net loading control.

Appendix I

Generation of phospho-specific antibodies against HPV-16 E7 S31S32

1. Design and synthesis of 2 peptides (1 non-phosphorylated + 1 phosphorylated)

The sequences of these peptides are as following below. The purity and quality of the peptides were verified using mass spectrometry.

Non-phosphorylated – H-CEQLNDSSEEED-NH₂

Phosphorylated – H-CEQLND S(PO₃H₂)S(PO₃H₂)EEED-NH₂

2. The phosphorylated peptide was coupled to a suitable carrier for immunization.
3. Two SPF rabbits were immunized with the phospho-peptide using a 28-day immunization schedule as shown in the schematic diagram.
Briefly, a sample of pre-immune sera was collected and the rabbit was then immunized with the phospho-peptide. On days 7, 10 and 18 a booster immunization was given to the animal. Then a medium bleed was collected on day 21 and final bleed was obtained on day 28.
4. The immune response was monitored by ELISA and the better-responding host was selected.
5. A double affinity purification of the phospho-antibody and non-phospho antibody was carried out as shown in the schematic diagram using phospho- and non-phospho-peptide columns. The purified antibodies were mixed with one volume of glycerol and stored at -20°C.
6. Finally, the specificity of the purified antibodies was further verified using indirect-ELISA and purity was analysed using SDS PAGE.

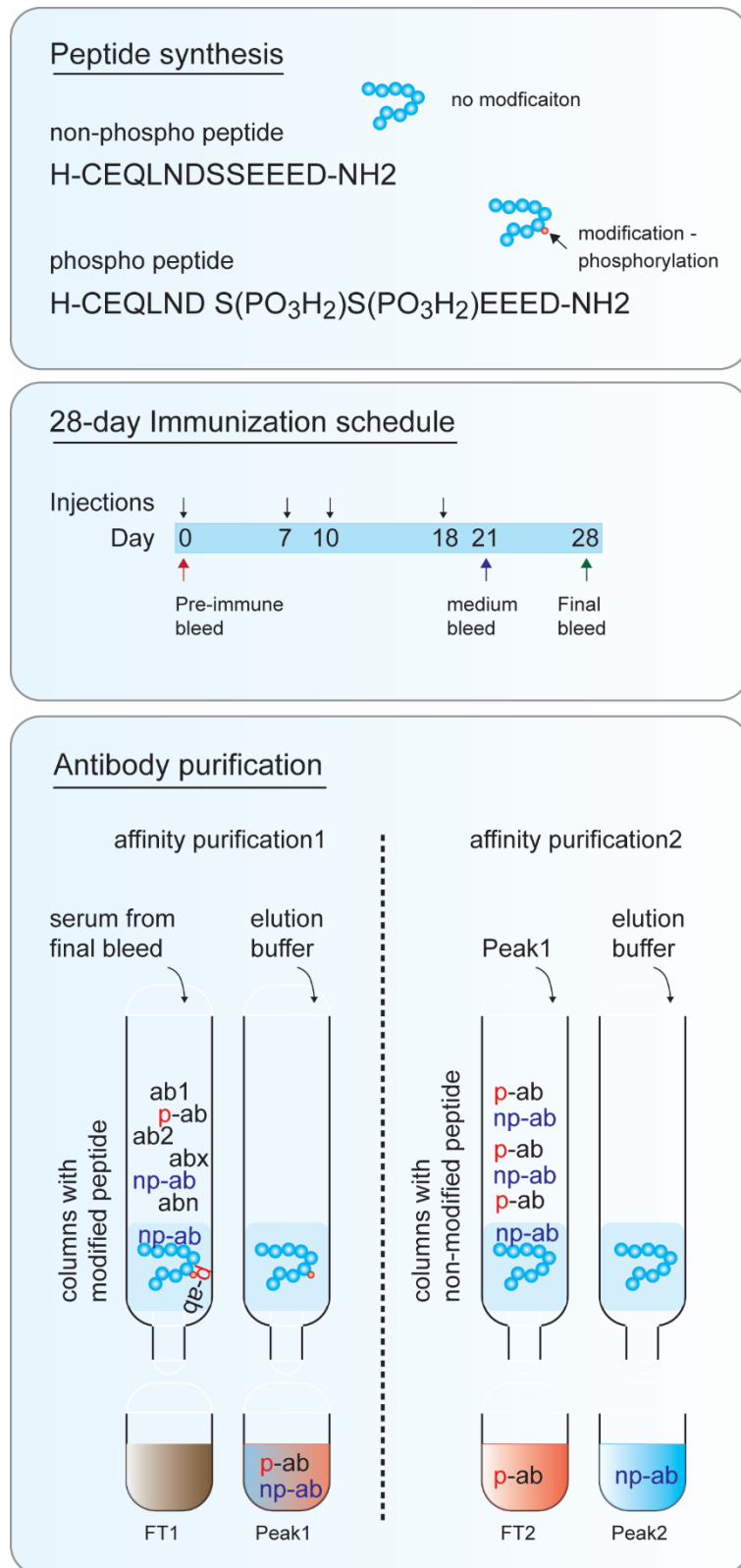


Figure 46. Generation of phospho-specific antibodies against HPV-16 E7 S31S32.

Appendix II

Full list of identified proteins pulled down ranked based on $\log(e)$ values (the base-10 log of the expectation that any particular protein assignment was made at random (E-value).)

1. Phospho-peptide
2. Non-phospho-peptide
3. Scrambled peptide

1. Phospho-peptide

rank	log(e)▲	log(I)	%/%	#	total	Mr	Accession	Description
1	-72.6	8.31	21/35	6	28	40.2	ENSP00000386101	ENSP00000386101 [18679/57102] RPL3:p, ribosomal protein L3 [Source:HGNC Symbol;Acc:HGNC:10332] IPR000597 Ribosomal protein L3 IPR009000 Translation protein, beta-barrel domain
2	-67.8	7.90	34/40	7	14	33.7	ENSP00000295065	ENSP00000295065 [1387/12366] MEMO1:p, mediator of cell motility 1 [Source: HGNC 14014] IPR002737 MEMO1 fam
3	-44.5	8.12	9.1/16	5	13	106.1	ENSP00000267163	ENSP00000267163 [2563/12915] RB1:p, retinoblastoma 1 [Source:HGNC Symbol;Acc:HGNC:9884] IPR013763 (x4) Cyclin-like IPR002720 Retinoblastoma-associated protein, A-box IPR002719 Retinoblastoma-associated protein, B-box IPR015030 Retinoblastoma-associated protein, C-terminal IPR024599 Retinoblastoma-associated protein, N-terminal
4	-38.8	7.78	11/17	4	6	59.7	ENSP00000358523	ENSP00000358523 [792/5102] VANGL1:p, VANGL planar cell polarity protein 1 [Source: HGNC 15512] IPR009539 (x2) Strabismus
5	-34.9	8.11	18/37	3	10	24.2	ENSP00000379888	ENSP00000379888 [33915/59432] RPS8:p, ribosomal protein S8 [Source:HGNC Symbol;Acc:HGNC:10441] IPR001047 Ribosomal protein S8e IPR018283 Ribosomal protein S8e, conserved site IPR022309 Ribosomal protein S8e/ribosomal biogenesis NSA2
6	-32.6	7.53	9.9/15	3	11	49.6	ENSP00000264071	ENSP00000264071 [75291/100508] TUBB4A:p, tubulin, beta 4A class IVa [Source:HGNC Symbol;Acc:HGNC:20774] IPR002452 (x8) Alpha tubulin IPR002453 (x13) Beta tubulin IPR002967 (x4) Delta tubulin IPR004057 (x6) Epsilon tubulin IPR002454 (x6) Gamma tubulin IPR019605 Misato Segment II tubulin-like domain IPR000217 (x9) Tubulin IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
7	-31.9	7.52	3.6/5	1	1	49.8	ENSP00000341289	(H) ENSP00000341289 [80543/105010] TUBB4B:p, tubulin, beta 4B class IVb [Source:HGNC Symbol;Acc:HGNC:20771] IPR002453 (x13) Beta tubulin IPR013838 Beta tubulin, autoregulation binding site IPR000217 (x9) Tubulin IPR017975 Tubulin, conserved site IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
8	-31.0	8.60	18/28	3	15	18.6	ENSP00000385621	ENSP00000385621 [23128/46075] RPL10:p, ribosomal protein L10 [Source:HGNC Symbol;Acc:HGNC:10298] IPR001197 Ribosomal protein L10e IPR016180 (x2) Ribosomal protein L10e/L16
9	-29.8	8.78	8.7/23	3	13	23.5	ENSP00000225430	ENSP00000225430 [18650/46654] RPL19:p, ribosomal protein L19 [Source:HGNC Symbol;Acc:HGNC:10312] IPR023638 Ribosomal protein L19/L19e conserved site IPR000196 Ribosomal protein L19/L19e domain
10	-27.6	7.57	6.8/11	3	10	62.3	ENSP00000252242	ENSP00000252242 [55012/97818] KRT5:p, keratin 5, type II [Source:HGNC Symbol;Acc:HGNC:6442] IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 Prefoldin
11	-26.9	7.29	5.7/11	2	3	59.3	ENSP00000253363	ENSP00000253363 [23189/43041] RBM39:p, RNA binding motif protein 39 [Source:HGNC Symbol;Acc:HGNC:15923] IPR000504 (x7) RNA recognition motif domain IPR003954 (x2) RNA recognition motif domain, eukaryote IPR029123 (x2) Splicing factor RBM39, linker IPR006509 Splicing factor, RBM39-like
12	-26.6	7.60	11/16	3	6	50.1	ENSP00000336799	ENSP00000336799 [87709/111896] TUBA1B:p, tubulin, alpha 1b [Source:HGNC Symbol;Acc:HGNC:18809] IPR002452 (x13) Alpha tubulin IPR000217 (x9) Tubulin IPR017975 Tubulin, conserved site IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
13	-26.5	7.35	10.0/12	3	4	51.5	ENSP00000167586	ENSP00000167586 [53489/94482] KRT14:p, keratin 14, type I [Source:HGNC Symbol;Acc:HGNC:6416] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
14	-23.2	7.68	11/17	3	4	47.7	ENSP00000311430	ENSP00000311430 [45215/62761] RPL4:p, ribosomal protein L4 [Source:HGNC Symbol;Acc:HGNC:10353] IPR025755 60S ribosomal protein L4, C-terminal domain IPR023574 Ribosomal protein L4 domain IPR002136 Ribosomal protein L4/L1e IPR013000 Ribosomal protein L4/L1e, eukaryotic/archaeal, conserved site
15	-21.9	7.23	10/16	2	9	32.7	ENSP00000202773	ENSP00000202773 [39760/58934] RPL6:p, ribosomal protein L6 [Source:HGNC Symbol;Acc:HGNC:10362] IPR000915 (x2) 60S ribosomal protein L6E IPR005568 Ribosomal protein L6, N-terminal IPR008991 Translation protein SH3-like domain
16	-21.9	7.85	19/38	2	6	15.4	ENSP00000361435	ENSP00000361435 [24332/45188] RPS24:p, ribosomal protein S24 [Source:HGNC Symbol;Acc:HGNC:10411]

[illegible]

									IPR009053 Prefoldin
									IPR010310 Type VII secretion system ESAT-6-like
35	-12.0	6.83	3.7/5	2	3	70.0	ENSP00000364802	ENSP00000364802 [84714/100968]	HSPA1A.p, heat shock 70kDa protein 1A [Source:HGNC Symbol;Acc:HGNC:5232]
									IPR013126 (x10) Heat shock protein 70 family
									IPR018181 (x3) Heat shock protein 70, conserved site
									IPR029048 Heat shock protein 70kD, C-terminal domain
									IPR029047 Heat shock protein 70kD, peptide-binding domain
36	-10.8	7.27	10/13	1	3	14.9	ENSP00000420311	ENSP00000420311 [38182/48173]	RPL23.p, ribosomal protein L23 [Source:HGNC Symbol;Acc:HGNC:10316]
									IPR023571 Ribosomal L14 dom
									IPR000218 Ribosomal L14b/L23e
37	-10.6	6.84	4.8/8	2	3	53.7	ENSP00000447566	ENSP00000447566 [75854/90399]	KRT8.p, keratin 8, type II [Source:HGNC Symbol;Acc:HGNC:6446]
									IPR001664 Intermediate filament protein
									IPR002957 (x5) Keratin, type I
									IPR003054 (x6) Keratin, type II
									IPR009053 Prefoldin
38	-10.5	7.31	7.0/12	1	3	21.4	ENSP0000039566	ENSP0000039566 [61092/72921]	HIST1H1C.p, histone cluster 1, H1c [Source:HGNC Symbol;Acc:HGNC:4716]
									IPR005819 (x7) Histone H5
									IPR005818 (x3) Linker histone H1/H5, domain H15
39	-10.2	7.04	17/35	1	6	14.0	ENSP00000332790	ENSP00000332790 [38468/61016]	HIST2H2AB.p, histone cluster 2, H2ab [Source:HGNC Symbol;Acc:HGNC:20508]
									IPR002119 (x7) Histone H2A
									IPR007125 Histone core
									IPR009072 Histone-fold
40	-8.5	8.35	14/32	1	10	14.1	ENSP00000358158	ENSP00000358158 [17262/23073]	HIST2H2AA3.p, histone cluster 2, H2aa3 [Source:HGNC Symbol;Acc:HGNC:4736]
									IPR003958 CBFA NFYB domain
									IPR009072 Histone-fold
									IPR002119 (x6) Histone H2A
									IPR007125 Histone core D
41	-8.0	6.66	5.0/9	2	2	58.2	ENSP00000411918	ENSP00000411918 [9605/14886]	PPAN-P2RY11.p, PPAN-P2RY11 readthrough [Source:HGNC Symbol;Acc:HGNC:33526]
									IPR004154 Anticodon-bd
									IPR007109 (x3) Brix
42	-7.9	7.69	10/20	1	5	14.5	ENSP00000264258	ENSP00000264258 [29948/40095]	RPL31.p, ribosomal protein L31 [Source:HGNC Symbol;Acc:HGNC:10394]
									IPR000054 Ribosomal protein L31e
									IPR020052 Ribosomal protein L31e, conserved site
43	-7.2	6.30	1.4/2	2	2	199.1	ENSP00000263994	ENSP00000263994 [7956/13483]	KIF1B.p, kinesin family member 1B [Source:HGNC Symbol;Acc:HGNC:16636]
									IPR000253 (x3) FHA dom
									IPR022140 KIF1B
									IPR022164 Kinesin-like
									IPR001752 (x7) Kinesin motor dom
									IPR027417 P-loop NTPase
									IPR001849 (x3) Pleckstrin homology
									IPR008984 SMAD FHA domain
44	-7.2	5.61	0.9/1	2	2	343.5	ENSP00000295550	ENSP00000295550 [27702/31026]	COL6A3.p, collagen, type VI, alpha 3 [Source:HGNC Symbol;Acc:HGNC:2213]
									IPR008160 (x2) Collagen
									IPR003961 (x2) Fibronectin type3
									IPR002223 (x7) Prot inh Kunz-m
									IPR002035 (x35) VWF A
45	-6.5	7.03	5.8/9	1	2	28.0	ENSP00000378378	ENSP00000378378 [43141/50433]	RPL8.p, ribosomal protein L8 [Source:HGNC Symbol;Acc:HGNC:10368]
									IPR012340 Nucleic acid-binding, OB-fold
									IPR022666 Ribosomal Proteins L2, RNA binding domain
									IPR002171 Ribosomal protein L2
									IPR022669 Ribosomal protein L2, C-terminal
									IPR022671 Ribosomal protein L2, conserved site
									IPR008991 Translation protein SH3-like domain
46	-6.5	6.47	3.2/5	1	1	49.6	ENSP00000339001	ENSP00000339001 [99182/105553]	TUBB.p, tubulin, beta class I [Source:HGNC Symbol;Acc:HGNC:20778]
									IPR002453 (x13) Beta tubulin
									IPR013838 Beta tubulin, autoregulation binding site
									IPR000217 (x9) Tubulin
									IPR017975 Tubulin, conserved site
									IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain
									IPR008280 Tubulin/FtsZ, C-terminal
									IPR003008 (x3) Tubulin/FtsZ, GTPase domain
47	-5.6	6.51	2.1/3	1	2	77.5	ENSP00000325376	ENSP00000325376 [66891/72273]	HNRNP.M.p, heterogeneous nuclear ribonucleoprotein M [Source:HGNC Symbol;Acc:HGNC:5046]
									IPR024666 Heterogeneous nuclear ribonucleoprotein M, PY nuclear localisation signal
									IPR000504 (x9) RNA recognition motif domain
48	-5.6	7.34	8.8/32	1	2	17.7	ENSP00000418868	ENSP00000418868 [32134/38118]	RPL29.p, ribosomal protein L29 [Source:HGNC Symbol;Acc:HGNC:10331]
									IPR002673 Ribosomal L29e
49	-5.2	6.98	11/18	1	3	11.4	ENSP00000376669	ENSP00000376669 [21152/23877]	HIST2H4A.p, histone cluster 2, H4a [Source:HGNC Symbol;Acc:HGNC:4794]
									IPR001951 Histone H4
									IPR007125 Histone core D
									IPR004823 TAF TATA-bd
50	-4.9	6.62	6.8/12	1	1	22.3	ENSP00000244534	ENSP00000244534 [65317/71527]	HIST1H1D.p, histone cluster 1, H1d [Source:HGNC Symbol;Acc:HGNC:4717]
									IPR005819 (x7) Histone H5
									IPR005818 (x2) Linker histone H1/H5, domain H15
51	-4.8	7.20	5.4/9	1	2	27.6	ENSP00000343557	ENSP00000343557 [8167/8963]	ZCC-HCL1.p, zinc finger, CCHC domain containing 17 [Source:HGNC Symbol;Acc:HGNC:30246]

									IPR012340 Nucleic acid-binding, OB-fold IPR022967 RNA-binding domain, S1 IPR003029 (x2) S1 domain IPR001878 Zinc finger, CCHC-type
52	-4.8	7.82	12/28	1	6	10.6	ENSP00000362671	ENSP00000362671 [26599/33623] RPL35.p, ribosomal protein L35 [Source:HGNC Symbol;Acc:HGNC:10344] IPR001854 (x3) Ribosomal protein L29 IPR018254 Ribosomal protein L29, conserved site	
53	-4.6	6.77	4.1/5	1	4	31.3	ENSP00000341885	ENSP00000341885 [53027/57545] RPS2.p, ribosomal protein S2 [Source:HGNC Symbol;Acc:HGNC:10404] IPR020568 Ribosomal protein S5 domain 2-type fold IPR005324 Ribosomal protein S5, C-terminal IPR013810 (x2) Ribosomal protein S5, N-terminal IPR018192 Ribosomal protein S5, N-terminal, conserved site IPR005711 Ribosomal protein S5, eukaryotic/archaeal	
54	-4.6	6.91	6.4/14	1	1	21.6	ENSP00000447001	ENSP00000447001 [46934/49916] RPL18.p, ribosomal protein L18 [Source:HGNC Symbol;Acc:HGNC:10310] IPR021132 Ribosomal protein L18e, conserved site IPR021131 (x2) Ribosomal protein L18e/L15P	
55	-4.5	6.56	7.6/16	1	2	17.8	ENSP00000377640	ENSP00000377640 [43704/47675] RPL24.p, ribosomal protein L24 [Source:HGNC Symbol;Acc:HGNC:10325] IPR023442 Ribosomal protein L24e, conserved site IPR000988 Ribosomal protein L24e-related IPR011017 TRASH domain	
56	-4.1	7.02	6.3/11	1	3	24.4	ENSP00000379731	ENSP00000379731 [50935/54405] RPL7.p, ribosomal protein L7 [Source:HGNC Symbol;Acc:HGNC:10363] IPR012988 Ribosomal protein L30, N-terminal IPR018038 Ribosomal protein L30, conserved site IPR016082 (x2) Ribosomal protein L30, ferredoxin-like fold domain IPR005998 Ribosomal protein L7, eukaryotic	
57	-3.9	5.68	1.7/2	1	1	122.3	ENSP00000327442	ENSP00000327442 [8949/10759] FMNL1.p,	
58	-3.6	7.51	15/29	1	2	15.8	ENSP00000253788	ENSP00000253788 [39320/42125] RPL27.p, ribosomal protein L27 [Source: HGNC 10328] IPR005824 KOW IPR001141 Ribosomal L27e	
59	-3.6	6.49	0.5/1	1	3	269.8	ENSP00000344789	ENSP00000344789 [7615/8603] ACACA.p, acetyl-CoA carboxylase alpha [Source: HGNC 84] IPR011761 ATP-grasp IPR003135 ATP-grasp carboxylate-amine IPR013537 AcCoA COase cen IPR005482 (x2) Biotin COase C IPR011764 Biotin carboxylation dom IPR000089 (x2) Biotin lipoyl IPR011763 COA CT C IPR011762 COA CT N IPR005481 CarbamoylP synth Isu N IPR000022 Carboxyl trans IPR005479 CbamoylP synth Isu-like ATP-bd IPR011095 Dala Dala lig C IPR016185 PreATP-grasp dom IPR011054 Rudment hybrid motif IPR011053 Single hybrid motif	
60	-3.3	6.98	5.7/9	1	2	24.2	ENSP00000307889	ENSP00000307889 [51523/54249] RPL13.p, ribosomal protein L13 [Source:HGNC Symbol;Acc:HGNC:10303] IPR001380 Ribosomal protein L13e IPR018256 Ribosomal protein L13e, conserved site	
61	-3.2	6.57	12/36	1	2	11.6	ENSP00000435188	ENSP00000435188 [10377/11481] AP001458.2.p, Uncharacterized protein	
62	-3.1	6.37	3.8/5	1	1	38.6	ENSP00000379342	ENSP00000379342 [77048/78643] ANKA2.p, annexin A2 [Source:HGNC Symbol;Acc:HGNC:537] IPR001464 (x6) Annexin IPR018502 (x8) Annexin repeat IPR002389 (x6) Annexin, type II IPR002392 (x4) Annexin, type V	
63	-3.0	6.96	18/40	1	2	10.3	ENSP00000418082	ENSP00000418082 [28128/29097] RPL37A.p, ribosomal protein L37a [Source:HGNC Symbol;Acc:HGNC:10348] IPR002674 (x2) Ribosomal protein L37ae IPR011332 Zinc-binding ribosomal protein	
64	-2.8	6.10	1.4/2	1	1	129.6	ENSP00000377527	ENSP00000377527 [24633/25260] PC.p, pyruvate carboxylase [Source:HGNC Symbol;Acc:HGNC:8636] IPR011761 ATP-grasp fold IPR005482 (x2) Biotin carboxylase, C-terminal IPR011764 Biotin carboxylation domain IPR000089 (x2) Biotin/lipoyl attachment IPR005481 Carbamoyl-phosphate synthase, large subunit, N-terminal IPR005479 Carbamoyl-phosphate synthetase large subunit-like, ATP-binding domain IPR003379 Carboxylase, conserved domain IPR011095 D-alanine--D-alanine ligase, C-terminal IPR016185 Pre-ATP-grasp domain IPR005930 (x2) Pyruvate carboxylase IPR000891 (x2) Pyruvate carboxyltransferase IPR011054 Rudiment single hybrid motif IPR011053 Single hybrid motif	
65	-2.5	7.22	7.8/11	1	3	22.8	ENSP00000248553	ENSP00000248553 [62569/64008] HSPB1.p, heat shock 27kDa protein 1 [Source:HGNC Symbol;Acc:HGNC:5246] IPR001436 (x7) Alpha crystallin/Heat shock protein IPR002068 (x2) Alpha crystallin/Hsp20 domain IPR008978 HSP20-like chaperone	
66	-2.4	6.22	9.0/22	1	1	17.2	ENSP00000428177	ENSP00000428177 [38697/39965] RPL26L1.p, ribosomal protein L26-like 1 [Source:HGNC Symbol;Acc:HGNC:17050] IPR005824 (x2) KOW IPR005825 Ribosomal protein L24/L26, conserved site	

									IPR005756 Ribosomal protein L26/L24P, eukaryotic/archaeal
									IPR008991 Translation protein SH3-like domain
67	-2.2	6.86	3.2/5	1	1	49.4	ENSP00000371894	ENSP00000371894 [28590/29036]	AP2M1:p, adaptor-related protein complex 2, mu 1 subunit [Source:HGNC Symbol;Acc:HGNC:564]
									IPR022775 AP complex, mu/sigma subunit
									IPR001392 (x7) Clathrin adaptor, mu subunit
									IPR008968 Clathrin adaptor, mu subunit, C-terminal
									IPR011012 Longin-like domain
									IPR028565 (x2) Mu homology domain
68	-2.2	7.21	9.6/18	1	3	15.8	ENSP00000416429	ENSP00000416429 [33265/33668]	RPL32:p, ribosomal protein L32 [Source:HGNC Symbol;Acc:HGNC:10336]
									IPR001515 Ribosomal protein L32e
									IPR018263 Ribosomal protein L32e, conserved site
69	-2.1	6.57	4.9/8	1	4	23.6	ENSP00000375730	ENSP00000375730 [45799/46230]	RPL13A:p, ribosomal protein L13a [Source:HGNC Symbol;Acc:HGNC:10304]
									IPR005822 Ribosomal protein L13
									IPR023564 Ribosomal protein L13 domain
									IPR023563 Ribosomal protein L13, conserved site
									IPR005755 Ribosomal protein L13, eukaryotic/archaeal
70	-2.0	6.58	2.0/3	1	3	62.0	ENSP00000395772	ENSP00000395772 [24929/25022]	GNL3:p, guanine nucleotide binding protein-like 3 (nucleolar) [Source:HGNC Symbol;Acc:HGNC:29931]
									IPR006073 GTP binding domain
									IPR014813 Guanine nucleotide-binding protein-like 3, N-terminal domain
									IPR027417 P-loop containing nucleoside triphosphate hydrolase
71	-2.0	6.25	5.2/8	1	1	27.7	ENSP00000258962	ENSP00000258962 [47462/47751]	SRSF1:p, serine/arginine-rich splicing factor 1 [Source:HGNC:10780]
									IPR000504 (x6) RRM dom
72	-2.0	6.27	16/26	1	1	15.5	ENSP00000346045	ENSP00000346045 [11437/11552]	RP517:p, ribosomal protein S17 [Source:HGNC:10397]
									IPR001210 (x2) Ribosomal S17e
73	-1.9	6.57	8.4/11	1	1	19.5	ENSP00000391278	ENSP00000391278 [37571/37681]	SRSF2:p, serine/arginine-rich splicing factor 2 [Source:HGNC:10783]
									IPR000504 RRM dom
74	-1.9	6.44	2.6/4	1	1	58.9	ENSP00000378360	ENSP00000378360 [23638/23731]	TKFC:p, dihydroxyacetone kinase 2 homolog (S. cerevisiae) [Source:HGNC:24552]
									IPR004006 Dak1
									IPR012734 Dhak ATP
									IPR004007 (x2) Dhak dom
75	-1.7	5.63	3.9/5	1	1	37.1	ENSP00000382507	ENSP00000382507 [1965/1982]	UBXN2B:p, UBX domain protein 2B [Source:HGNC:27035]
									IPR012989 (x3) SEP domain
									IPR001012 (x3) UBX
76	-1.7	6.70	2.2/5	1	1	64.8	ENSP00000384979	ENSP00000384979 [19/32]	LINGO3:p,
77	-1.7	6.33	7.1/13	1	1	25.0	ENSP00000302111	ENSP00000302111 [9804/9843]	MGMT:p, O-6-methylguanine-DNA methyltransferase [Source:HGNC:7059]
									IPR014048 (x3) MethylDNA cys MeTrfase DNA-bd
									IPR008332 MethylG MeTrfase
78	-1.6	5.01	6.1/11	1	1	34.8	ENSP00000377545	ENSP00000377545 [142/160]	CLQTNF2:p,
79	-1.4	6.53	13/43	1	1	16.3	ENSP00000385958	ENSP00000385958 [54387/54448]	RPS14:p, ribosomal protein S14 [Source:HGNC Symbol;Acc:HGNC:10387]
									IPR018102 Ribosomal S11, conserved site
									IPR001971 (x2) Ribosomal protein S11
80	-1.4	5.89	0.8/2	1	1	209.3	ENSP00000264382	ENSP00000264382 [1870/1936]	Sl:p, sucrase-isomaltase (alpha-glucosidase) [Source:HGNC:10856]
									IPR011013 (x2) Gal mutarotase SF dom
									IPR000322 (x2) Glyco hydro 31
									IPR017853 (x2) Glycoside hydrolase SF
									IPR000519 (x4) P trefoil
81	-1.3	5.00	2.5/4	1	1	48.9	ENSP00000261625	ENSP00000261625 [28/34]	no protein information available
82	-1.3	5.25	2.7/5	1	1	75.2	ENSP00000376989	ENSP00000376989 [4616/4630]	ZNF800:p, zinc finger protein 800 [Source:HGNC:27267]
									IPR007087 (x4) Znf C2H2
									IPR015880 (x7) Znf C2H2-like
83	-1.3	7.55	2.2/3	1	1	85.6	ENSP00000362924	ENSP00000362924 [57498/57584]	GSN:p,
84	-1.3	7.51	2.2/3	1	2	69.6	ENSP00000171111	ENSP00000171111 [6788/6823]	KEAP1:p,

8.1/14.5 #27 #20

2. non-phospho-peptide

rank	log(e) [▲]	log(l)	%/%	#	total	Mr	Accession	Description
1	-79.7	8.36	35/40	7	34	33.7	ENSP00000295065	ENSP00000295065 [1065/12366] MEMO1.p, mediator of cell motility 1 [Source:HGNC Symbol;Acc:HGNC:14014] IPR002737 MEMO1 fam
2	-47.7	7.91	8.9/16	5	10	106.1	ENSP00000267163	ENSP00000267163 [2385/12915] RB1.p, retinoblastoma 1 [Source:HGNC Symbol;Acc:HGNC:9884] IPR013763 (x4) Cyclin-like IPR002720 Retinoblastoma-associated protein, A-box IPR002719 Retinoblastoma-associated protein, B-box IPR015030 Retinoblastoma-associated protein, C-terminal IPR024599 Retinoblastoma-associated protein, N-terminal
3	-39.8	7.41	12/15	4	12	51.5	ENSP00000167586	ENSP00000167586 [44632/94482] KRT14.p, keratin 14, type I [Source:HGNC Symbol;Acc:HGNC:6416] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
4	-32.4	7.11	10/18	3	6	46.5	ENSP00000347005	ENSP00000347005 [17617/38257] LUC7L2.p, C7orf55-LUC7L2 readthrough [Source:HGNC Symbol;Acc:HGNC:44671] IPR004882 Luc7-related
5	-31.6	7.36	17/19	4	8	44.1	ENSP00000355124	ENSP00000355124 [36380/83009] KRT19.p, keratin 19, type I [Source:HGNC Symbol;Acc:HGNC:6436] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
6	-27.4	7.02	9.1/17	3	5	59.3	ENSP00000253363	ENSP00000253363 [23014/43041] RBM39.p, RNA binding motif protein 39 [Source:HGNC Symbol;Acc:HGNC:15923] IPR000504 (x7) RNA recognition motif domain IPR003954 (x2) RNA recognition motif domain, eukaryote IPR029123 (x2) Splicing factor RBM39, linker IPR006509 Splicing factor, RBM39-like
7	-27.4	7.20	6.4/10	3	9	62.3	ENSP00000252242	ENSP00000252242 [55196/97818] KRT5.p, keratin 5, type II [Source:HGNC Symbol;Acc:HGNC:6442] IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 Prefoldin
8	-27.2	7.14	11/16	3	4	50.1	ENSP00000336799	ENSP00000336799 [87316/111896] TUBA1B.p, tubulin, alpha 1b [Source:HGNC Symbol;Acc:HGNC:18809] IPR002452 (x13) Alpha tubulin IPR000217 (x9) Tubulin IPR017975 Tubulin, conserved site IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
9	-22.5	6.95	19/45	2	4	18.6	ENSP00000346027	ENSP00000346027 [24183/43302] RPL21.p, ribosomal protein L21 [Source:HGNC Symbol;Acc:HGNC:10313] IPR001147 Ribosomal protein L21e IPR018259 Ribosomal protein L21e, conserved site IPR008991 Translation protein SH3-like domain
10	-21.3	7.61	12/24	2	7	19.3	ENSP00000362820	ENSP00000362820 [27100/49326] SRSF3.p, serine/arginine-rich splicing factor 3 [Source:HGNC Symbol;Acc:HGNC:10785] IPR000504 (x3) RNA recognition motif domain
11	-19.0	7.52	8.4/16	1	1	27.4	ENSP00000325905	(H) ENSP00000325905 [22639/43818] SRSF7.p, serine/arginine-rich splicing factor 7 [Source:HGNC Symbol;Acc:HGNC:10789] IPR000504 (x3) RNA recognition motif domain IPR001878 (x2) Zinc finger, CCHC-type
12	-18.0	7.09	3.4/6	2	3	66.0	ENSP00000252244	ENSP00000252244 [104396/128657] KRT1.p, keratin 1, type II [Source:HGNC Symbol;Acc:HGNC:6412] IPR001664 Intermediate filament protein IPR018039 Intermediate filament protein, conserved site IPR003054 (x6) Keratin, type II
13	-17.8	7.32	12/25	2	5	24.2	ENSP00000379888	ENSP00000379888 [43518/59432] RPS8.p, ribosomal protein S8 [Source:HGNC Symbol;Acc:HGNC:10441] IPR001047 Ribosomal protein S8e IPR018283 Ribosomal protein S8e, conserved site IPR022309 Ribosomal protein S8e/ribosomal biogenesis NSA2
14	-15.3	7.09	15/25	2	5	21.4	ENSP00000397798	ENSP00000397798 [17724/26721] ribosomal protein L17 [Source:HGNC:10307] IPR001063 Ribosomal L22 IPR005721 Ribosomal L22/L17 euk/arc
15	-15.0	6.91	7.2/8	2	2	43.7	ENSP00000447278	ENSP00000447278 [41024/60920] KRT18.p, keratin 18, type I [Source:HGNC Symbol;Acc:HGNC:6430] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I
16	-13.9	6.72	11/18	2	2	40.2	ENSP00000386101	ENSP00000386101 [42250/57102] RPL3.p, ribosomal protein L3 [Source:HGNC Symbol;Acc:HGNC:10332] IPR000597 Ribosomal protein L3 IPR009000 Translation protein, beta-barrel domain
17	-13.4	7.04	14/30	2	3	25.5	ENSP00000376276	ENSP00000376276 [26108/38531] SRSF2.p, serine/arginine-rich splicing factor 2 [Source:HGNC Symbol;Acc:HGNC:10783] IPR000504 (x3) RNA recognition motif domain IPR003954 RNA recognition motif domain, eukaryote
18	-13.3	7.10	8.2/22	1	3 _k	23.5	ENSP00000225430	ENSP00000225430 [33676/46654] RPL19.p, ribosomal protein L19 [Source:HGNC Symbol;Acc:HGNC:10312] IPR023638 Ribosomal protein L19/L19e conserved site IPR000196 Ribosomal protein L19/L19e domain
19	-12.1	8.02	5.6/9	1	9	24.6	ENSP00000358832	ENSP00000358832 [37111/48063] RPL10.p, ribosomal protein L10 [Source:HGNC Symbol;Acc:HGNC:10298] IPR001197 (x2) Ribosomal protein L10e

20	-11.5	6.92	17/35	1	7	14.0	ENSP00000332790	IPR018255 Ribosomal protein L10e, conserved site IPR016180 (x2) Ribosomal protein L10e/L16 ENSP00000332790 [36776/61016] HIST2H2AB:p, histone cluster 2, H2ab [Source:HGNC Symbol;Acc:HGNC:20508] IPR002119 (x7) Histone H2A IPR007125 Histone core IPR009072 Histone-fold
21	-10.4	6.84	2.5/3	1	1	48.1	ENSP00000308452	(H) ENSP00000308452 [58087/80352] KRT17:p, keratin 17, type I [Source:HGNC Symbol;Acc:HGNC:6427] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
22	-10.3	7.61	11/15	1	9	13.9	ENSP00000358164	ENSP00000358164 [70310/84130] HIST2H2BF:p, histone cluster 2, H2bf [Source:HGNC Symbol;Acc:HGNC:24700] IPR000558 (x6) Histone H2B IPR007125 Histone core IPR009072 Histone-fold IPR009358 Transcription factor CBF/NF- κ /archaeal histone
23	-10.2	7.03	2.8/4	1	2	60.0	ENSP00000252250	ENSP00000252250 [84375/102910] KRT6C:p, keratin 6C, type II [Source:HGNC Symbol;Acc:HGNC:20406] IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 (x2) Prefoldin
24	-9.6	7.52	5.6/15	1	6	28.7	ENSP00000369757	ENSP00000369757 [42458/51983] RPS6:p, ribosomal protein S6 [Source:HGNC Symbol;Acc:HGNC:10429] IPR014401 Ribosomal protein S6, eukaryotic IPR001377 Ribosomal protein S6e IPR018282 Ribosomal protein S6e, conserved site
25	-9.5	7.22	5.4/9	1	2	27.6	ENSP00000343557	ENSP00000343557 [6814/8983] ZCCHC17:p, zinc finger, CCHC domain containing 17 [Source:HGNC Symbol;Acc:HGNC:30246] IPR012340 Nucleic acid-binding, OB-fold IPR022967 RNA-binding domain, S1 IPR003029 (x2) S1 domain IPR001878 Zinc finger, CCHC-type
26	-8.9	6.36	2.6/4	2	3	104.5	ENSP00000262325	ENSP00000262325 [11059/14668] AP2B1:p, adaptor-related protein complex 2, beta 1 subunit [Source: HGNC 563] IPR016342 AP complex bsu 1 2 4 IPR016024 ARM-type fold IPR000225 (x2) Armadillo IPR015151 B-adaptin app sub C IPR002553 Clathrin/coatomer adapt-like N IPR008152 (x2) Clathrin a/b/g-adaptin app lg IPR009028 Coatomer/calthrin app sub C IPR013041 Coatomer/clathrin app lg-like IPR000357 HEAT
27	-8.7	6.17	11/17	2 _{kl}	2	53.7	ENSP00000447566	ENSP00000447566 [78245/90399] KRT8:p, keratin 8, type II [Source:HGNC Symbol;Acc:HGNC:6446] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR003054 (x6) Keratin, type II IPR009053 Prefoldin
28	-8.5	6.34	2.6/3	1	1	49.6	ENSP00000246635	(H) ENSP00000246635 [56901/78257] KRT13:p, keratin 13 [Source:HGNC Symbol;Acc:HGNC:6415] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
29	-8.4	6.99	3.1/5	1	1	49.9	ENSP00000248437	(H) ENSP00000248437 [91988/104093] TUBA4A:p, tubulin, alpha 4a [Source:HGNC Symbol;Acc:HGNC:12407] IPR002452 (x13) Alpha tubulin IPR002453 (x7) Beta tubulin IPR002967 (x4) Delta tubulin IPR004057 (x7) Epsilon tubulin IPR000217 (x9) Tubulin IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
30	-7.8	7.63	7.3/14	1	2	20.2	ENSP00000363676	ENSP00000363676 [44121/53203] RPL11:p, ribosomal protein L11 [Source:HGNC Symbol;Acc:HGNC:10301] IPR002132 Ribosomal protein L5 IPR022803 Ribosomal protein L5 domain IPR031309 Ribosomal protein L5, C-terminal IPR031310 Ribosomal protein L5, N-terminal IPR020929 Ribosomal protein L5, conserved site
31	-7.5	6.96	12/28	1	3	10.6	ENSP00000362671	ENSP00000362671 [22139/33623] RPL35:p, ribosomal protein L35 [Source:HGNC Symbol;Acc:HGNC:10344] IPR001854 (x3) Ribosomal protein L29 IPR018254 Ribosomal protein L29, conserved site
32	-7.5	6.16	5.7/9	2	2	22.6	ENSP00000302896	ENSP00000302896 [40102/47879] RPS9:p, ribosomal protein S9 [Source:HGNC Symbol;Acc:HGNC:10442] IPR002942 (x3) RNA-binding S4 domain IPR018079 Ribosomal protein S4, conserved site IPR001912 Ribosomal protein S4/S9, N-terminal IPR005710 Ribosomal protein S4/S9, eukaryotic/archaeal
33	-7.4	7.53	14/32	1	8	14.1	ENSP00000358158	ENSP00000358158 [17884/23073] HIST2H2AA3:p, histone cluster 2, H2aa3 [Source: HGNC 4736] IPR003958 CBFA NFYB domain IPR009072 Histone-fold IPR002119 (x6) Histone H2A IPR007125 Histone core D
34	-7.4	6.65	11/22	1	1	15.4	ENSP00000361435	ENSP00000361435 [36218/45188] RPS24:p, ribosomal protein S24 [Source:HGNC Symbol;Acc:HGNC:10411] IPR018098 Ribosomal S24e conserved site

35	-7.0	5.97	3.0/5	2	2	105.7	ENSP00000356290	IPR012678 Ribosomal protein L23/L15e core domain IPR001976 Ribosomal protein S24e ENSP00000356290 [15760/20566] MTHFD1L.p, methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1-like [Source: HGNC:21055] IPR000559 Formate THF ligase IPR027417 P-loop NTPase IPR000672 (x4) THF DH/CycHdriase IPR020631 THF DH/CycHdriase NAD-bd dom IPR020630 THF DH/CycHdriase cat dom
36	-6.3	6.41	7.1/13	1	2	23.7	ENSP00000441406	ENSP00000441406 [14011/17004] ARL6IP4.p,
37	-6.2	6.55	12/22	1	2	13.0	ENSP00000348849	ENSP00000348849 [30809/38297] RPS26.p, ribosomal protein S26 [Source:HGNC Symbol;Acc:HGNC:10414] IPR000892 (x2) Ribosomal protein S26e
38	-5.4	6.17	8.8/32	1	2	17.7	ENSP00000418868	ENSP00000418868 [32733/38118] RPL29.p, ribosomal protein L29 [Source: HGNC:10331] IPR002673 Ribosomal L29e
39	-5.1	7.06	10/20	1	4	14.5	ENSP00000264258	ENSP00000264258 [33673/40095] RPL31.p, ribosomal protein L31 [Source:HGNC Symbol;Acc:HGNC:10334] IPR000054 Ribosomal protein L31e IPR020052 Ribosomal protein L31e, conserved site
40	-4.7	6.22	3.2/5	1	2	49.6	ENSP00000339001	ENSP00000339001 [101211/105553] TUBB.p, tubulin, beta class I [Source:HGNC Symbol;Acc:HGNC:20778] IPR002453 (x13) Beta tubulin IPR013838 Beta tubulin, autoregulation binding site IPR000217 (x9) Tubulin IPR017975 Tubulin, conserved site IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
41	-4.6	5.62	1.8/3	1	1	103.9	ENSP00000413234	ENSP00000413234 [31171/35630] AP2A2.p, adaptor-related protein complex 2, alpha 2 subunit [Source:HGNC Symbol;Acc:HGNC:562] IPR017104 Adaptor protein complex AP-2, alpha subunit IPR016024 Armadillo-type fold IPR003164 Clathrin adaptor, alpha-adaptin, appendage, C-terminal subdomain IPR008152 (x2) Clathrin adaptor, alpha/beta/gamma-adaptin, appendage, Ig-like subdomain IPR002553 Clathrin/coatomer adaptor, adaptin-like, N-terminal IPR009028 Coatomer/calthrin adaptor appendage, C-terminal subdomain IPR013041 Coatomer/clathrin adaptor appendage, Ig-like subdomain
42	-4.5	6.53	4.0/5	1	2	42.0	ENSP00000355645	ENSP00000355645 [121117/127030] ACTA1.p, actin, alpha 1, skeletal muscle [Source:HGNC Symbol;Acc:HGNC:129] IPR004000 (x8) Actin family IPR004001 (x2) Actin, conserved site IPR020902 Actin/actin-like conserved site
43	-4.2	6.59	7.7/17	1	1	17.7	ENSP00000389103	ENSP00000389103 [45001/48379] RPL23A.p, ribosomal protein L23a [Source:HGNC Symbol;Acc:HGNC:10317] IPR019985 Ribosomal protein L23 IPR012678 Ribosomal protein L23/L15e core domain IPR005633 Ribosomal protein L23/L25, N-terminal IPR001014 Ribosomal protein L23/L25, conserved site IPR013025 Ribosomal protein L25/L23
44	-4.2	5.81	5.6/12	1	1	35.9	ENSP00000361626	ENSP00000361626 [58808/61179] YBX1.p, Y box binding protein 1 [Source:HGNC Symbol;Acc:HGNC:8014] IPR011129 Cold shock protein IPR019844 Cold-shock conserved site IPR002059 (x4) Cold-shock protein, DNA-binding IPR012940 Nucleic acid-binding, OB-fold
45	-4.0	5.84	2.1/3	1	1	62.0	ENSP00000246662	ENSP00000246662 [101155/106151] KRT9.p, keratin 9, type I [Source:HGNC Symbol;Acc:HGNC:6447] IPR001664 Intermediate filament protein IPR018039 Intermediate filament protein, conserved site IPR002957 (x5) Keratin, type I
46	-3.9	6.61	11/18	1	2	11.4	ENSP00000376669	ENSP00000376669 [22106/23877] HIST2H4A.p, histone cluster 2, H4a [Source: HGNC:4794] IPR001951 Histone H4 IPR007125 Histone core D IPR004823 TAF TATA-bd
47	-3.4	5.80	5.2/11	1	1	33.6	ENSP00000416959	ENSP00000416959 [32777/34429] TRA2B.p, transformer 2 beta homolog (Drosophila) [Source:HGNC Symbol;Acc:HGNC:10781] IPR000504 (x3) RNA recognition motif domain
48	-3.2	6.04	4.9/8	1	1	23.6	ENSP00000375730	ENSP00000375730 [44209/46230] RPL13A.p, ribosomal protein L13a [Source:HGNC Symbol;Acc:HGNC:10304] IPR005822 Ribosomal protein L13 IPR023564 Ribosomal protein L13 domain IPR023563 Ribosomal protein L13, conserved site IPR005755 Ribosomal protein L13, eukaryotic/archaeal
49	-2.8	6.49	4.9/7	1	1	29.4	ENSP00000272139	ENSP00000272139 [14984/15664] Clorf35.p, chromosome 1 open reading frame 35 [Source:HGNC Symbol;Acc:HGNC:19032] IPR019315 Kinase phosphorylation domain
50	-2.4	5.92	4.9/8	1	1	32.7	ENSP00000202773	ENSP00000202773 [57921/58934] RPL6.p, ribosomal protein L6 [Source:HGNC Symbol;Acc:HGNC:10362] IPR000915 (x2) 60S ribosomal protein L6E IPR005568 Ribosomal protein L6, N-terminal IPR008991 Translation protein SH3-like domain
51	-2.2	6.18	6.6/11	1	1	22.6	ENSP00000330074	ENSP00000330074 [51816/52583] HIST1H1B.p, histone cluster 1, H1b [Source:HGNC Symbol;Acc:HGNC:4719] IPR005819 (x6) Histone H5 IPR005818 (x3) Linker histone H1/H5, domain H15
52	-2.0	6.24	6.0/10	1	1	27.7	ENSP00000258962	ENSP00000258962 [47462/47751]

									SRSF1:p, serine/arginine-rich splicing factor 1 [Source: HGNC 10780] IPR000504 (x6) RRM dom
53	-1.9	5.84	7.8/11	1	1	22.8	ENSP00000248553	ENSP00000248553 [63692/64008] HSPB1:p, heat shock 27kDa protein 1 [Source:HGNC Symbol;Acc:HGNC:5246] IPR001436 (x7) Alpha crystallin/Heat shock protein IPR002068 (x2) Alpha crystallin/Hsp20 domain IPR008978 HSP20-like chaperone	
54	-1.9	6.53	15/29	1	1	15.8	ENSP00000253788	ENSP00000253788 [41803/42125] RPL27:p, ribosomal protein L27 [Source: HGNC 10328] IPR005824 KOW IPR001141 Ribosomal L27e	
55	-1.9	6.41	2.6/4	1	1	58.9	ENSP00000378360	ENSP00000378360 [23638/23731] TKFC:p, dihydroxyacetone kinase 2 homolog (S. cerevisiae) [Source: HGNC 24552] IPR004006 Dak1 IPR012734 Dhak ATP IPR004007 (x2) DhaL dom	
56	-1.7	5.58	1.8/3	1	1	125.3	ENSP00000359027	ENSP00000359027 [3499/3536] SENP6:p,	
57	-1.6	6.81	9.6/18	1	2	15.8	ENSP00000416429	ENSP00000416429 [33606/33668] RPL32:p, ribosomal protein L32 [Source:HGNC Symbol;Acc:HGNC:10336] IPR001515 Ribosomal protein L32e IPR018263 Ribosomal protein L32e, conserved site	
58	-1.6	5.74	2.5/6	1	1	44.7	ENSP00000431296	ENSP00000431296 [5830/5850] CD36:p,	
59	-1.5	5.53	7.0/13	1	1	15.8	ENSP00000296674	ENSP00000296674 [45755/45831] RPS23:p, ribosomal protein S23 [Source:HGNC Symbol;Acc:HGNC:10410] IPR012340 Nucleic acid-binding, OB-fold IPR006032 (x3) Ribosomal protein S12/S23 IPR005680 Ribosomal protein S23, eukaryotic/archaeal	
60	-1.5	5.51	6.4/14	1	1	21.6	ENSP00000447001	ENSP00000447001 [49876/49916] RPL18:p, ribosomal protein L18 [Source:HGNC Symbol;Acc:HGNC:10310] IPR021132 Ribosomal protein L18e, conserved site IPR021131 (x2) Ribosomal protein L18e/L15P	
61	-1.5	5.29	11/15	1	1	11.3	ENSP00000456484	ENSP00000456484 [35/39] CCDC154:p,	
62	-1.5	6.21	1.2/2	1	1	91.9	ENSP00000453583	ENSP00000453583 [610/625] RASGRP1:p,	
63	-1.4	5.83	1.9/3	1	1	58.8	ENSP00000269576	ENSP00000269576 [111987/112614] KRT10:p, keratin 10, type I [Source:HGNC Symbol;Acc:HGNC:6413] IPR001664 Intermediate filament protein IPR018039 Intermediate filament protein, conserved site IPR002957 (x5) Keratin, type I IPR009053 Prefoldin	
64	-1.4	6.76	2.2/3	1	2	69.6	ENSP00000171111	ENSP00000171111 [6784/6823] KEAP1:p,	
65	-1.4	6.15	5.7/9	1	1	24.2	ENSP00000307889	ENSP00000307889 [54076/54249] RPL13:p, ribosomal protein L13 [Source:HGNC Symbol;Acc:HGNC:10303] IPR001380 Ribosomal protein L13e IPR018256 Ribosomal protein L13e, conserved site	
66	-1.3	4.95	0.8/1	1	1	149.7	ENSP00000370257	ENSP00000370257 [272/331] FREM1:p,	
67	-1.3	5.84	2.1/3	1	1	49.8	ENSP00000400852	ENSP00000400852 [811/819] ZNF736:p,	
68	-1.3	7.26	6.5/10	1	1	38.1	ENSP00000368447	ENSP00000368447 [7842/7867] GPALPP1:p, GPALPP motifs containing 1 [Source: HGNC 20298] IPR022226 DUF3752	
69	-1.3	5.18	3.0/4	1	1	27.9	ENSP00000303963	ENSP00000303963 [1791/1882] CTRB2:p, chymotrypsinogen B2 [Source: HGNC 2522] IPR001254 (x3) Peptidase S1 IPR001314 (x3) Peptidase S1A IPR009003 Trypsin-like Pept dom	

6.9/12.3 #27 #18

3. Scrambled peptide

rank	log(e)▲	log(l)	%/%	#	total	Mr	Accession	Description
1	-85.1	7.80	28/32	7	17	44.1	ENSP00000355124	ENSP00000355124 ●● KRT19:p, keratin 19, type I [Source:HGNC Symbol;Acc:HGNC:6436] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
2	-48.8	7.59	21/25	5	14	48.0	ENSP00000373487	ENSP00000373487 ●● KRT18:p, keratin 18, type I [Source:HGNC Symbol;Acc:HGNC:6430] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I
3	-44.0	7.42	12/15	4	14	51.5	ENSP00000167586	ENSP00000167586 ●● KRT14:p, keratin 14, type I [Source:HGNC Symbol;Acc:HGNC:6416] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
4	-39.3	7.36	14/21	4	5	50.1	ENSP00000336799	ENSP00000336799 ●● TUBA1B:p, tubulin, alpha 1b [Source:HGNC Symbol;Acc:HGNC:18809] IPR002452 (x13) Alpha tubulin IPR000217 (x9) Tubulin IPR017975 Tubulin, conserved site IPR018316 (x2) Tubulin/FtsZ, 2-layer sandwich domain IPR008280 Tubulin/FtsZ, C-terminal IPR003008 (x3) Tubulin/FtsZ, GTPase domain
5	-37.7	7.26	10/13	4	7	65.4	ENSP00000310861	ENSP00000310861 ●● KRT2:p, keratin 2, type II [Source:HGNC Symbol;Acc:HGNC:6439] IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 Prefoldin IPR010310 Type VII secretion system ESAT-6-like
6	-35.6	7.60	8.5/16	3	8	59.3	ENSP00000253363	ENSP00000253363 ●● RBM9:p, RNA binding motif protein 39 [Source:HGNC Symbol;Acc:HGNC:15923] IPR000504 (x7) RNA recognition motif domain IPR003954 (x2) RNA recognition motif domain, eukaryote IPR029123 (x2) Splicing factor RBM9, linker IPR006509 Splicing factor, RBM39-like
7	-35.4	7.34	5.1/9	3	6	66.0	ENSP00000252244	(H) ENSP00000252244 ●● KRT1:p, keratin 1, type II [Source:HGNC Symbol;Acc:HGNC:6412] IPR001664 Intermediate filament protein IPR018039 Intermediate filament protein, conserved site IPR003054 (x6) Keratin, type II
8	-31.3	7.13	7.1/13	2	5	46.5	ENSP00000347005	ENSP00000347005 ●● LUC7L2:p, C7orf55-LUC7L2 readthrough [Source:HGNC Symbol;Acc:HGNC:44671] IPR004882 Luc7-related
9	-30.7	7.51	4.9/8	2	7	62.3	ENSP00000252242	(H) ENSP00000252242 ●● KRT5:p, keratin 5, type II [Source:HGNC Symbol;Acc:HGNC:6442] IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 Prefoldin
10	-26.7	6.91	8.6/10	2	2	49.2	ENSP00000254043	(H) ENSP00000254043 ●● KRT15:p, keratin 15 [Source:HGNC Symbol;Acc:HGNC:6421] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
11	-26.1	8.33	19/37	3	13	19.3	ENSP00000362820	ENSP00000362820 ●● SRF3:p, serine/arginine-rich splicing factor 3 [Source:HGNC Symbol;Acc:HGNC:10785] IPR000504 (x3) RNA recognition motif domain
12	-24.0	7.89	15/24	3	14	27.7	ENSP00000258962	ENSP00000258962 ●● SRF1:p, serine/arginine-rich splicing factor 1 [Source:HGNC:10780] IPR000504 (x6) RRM dom
13	-23.4	7.56	7.2/11	3	10	51.4	ENSP00000329243	ENSP00000329243 ●● KRT7:p, keratin 7, type II [Source:HGNC Symbol;Acc:HGNC:6445] IPR020822 Chorismate mutase, type II IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 Prefoldin
14	-23.0	8.01	11/21	2	3	27.4	ENSP00000325905	(H) ENSP00000325905 ●● SRF7:p, serine/arginine-rich splicing factor 7 [Source:HGNC Symbol;Acc:HGNC:10789] IPR000504 (x3) RNA recognition motif domain IPR001878 (x2) Zinc finger, CCHC-type
15	-21.3	7.05	2.8/4	1	2	49.6	ENSP00000246635	(H) ENSP00000246635 ●● KRT13:p, keratin 13 [Source:HGNC Symbol;Acc:HGNC:6415] IPR001664 Intermediate filament protein IPR002957 (x5) Keratin, type I IPR009053 Prefoldin
16	-20.9	7.26	2.8/4	1	3	60.0	ENSP00000252250	(H) ENSP00000252250 ●● KRT6C:p, keratin 6C, type II [Source:HGNC Symbol;Acc:HGNC:20406] IPR001664 Intermediate filament protein IPR003054 (x6) Keratin, type II IPR009053 (x2) Prefoldin
17	-20.8	7.60	9.7/20	2	11	33.6	ENSP00000416959	ENSP00000416959 ●● TRA2B:p, transformer 2 beta homolog [Drosophila] [Source:HGNC Symbol;Acc:HGNC:10781] IPR000504 (x3) RNA recognition motif domain
18	-20.0	7.52	14/30	2	13%	25.5	ENSP00000376276	ENSP00000376276 ●● SRF2:p, serine/arginine-rich splicing factor 2 [Source:HGNC Symbol;Acc:HGNC:10783] IPR000504 (x3) RNA recognition motif domain IPR003954 RNA recognition motif domain, eukaryote
19	-20.0	6.49	5.6/9	2	3	62.0	ENSP00000246662	ENSP00000246662 ●● KRT9:p, keratin 9, type I [Source:HGNC Symbol;Acc:HGNC:6447]

125

									IPR023638 Ribosomal protein L19/L19e conserved site IPR000196 Ribosomal protein L19/L19e domain
40	-4.7	6.79	7.3/14	1	2	20.2	ENSP00000363676	ENSP00000363676	RPL11:p, ribosomal protein L11 [Source:HGNC Symbol;Acc:HGNC:10301] IPR002132 Ribosomal protein L5 IPR022803 Ribosomal protein L5 domain IPR031309 Ribosomal protein L5, C-terminal IPR031310 Ribosomal protein L5, N-terminal IPR020929 Ribosomal protein L5, conserved site
41	-4.3	6.31	3.0/5	1	1	47.7	ENSP00000311430	ENSP00000311430	RPL4:p, ribosomal protein L4 [Source:HGNC Symbol;Acc:HGNC:10353] IPR025755 60S ribosomal protein L4, C-terminal domain IPR023574 Ribosomal protein L4 domain IPR002136 Ribosomal protein L4/L1e IPR013000 Ribosomal protein L4/L1e, eukaryotic/archaea, conserved site
42	-3.8	6.44	1.6/2	1	1	82.5	ENSP00000381293	ENSP00000381293	NSF:p, N-ethylmaleimide-sensitive factor [Source: HGNC 8016] IPR003593 AAA+ ATPase IPR013093 ATPase AAA-2 IPR003959 (x2) ATPase AAA core IPR009010 Asp de-COase-like dom IPR003338 CD4 N-term subdom IPR004201 Cdc48 dom2 IPR027417 (x2) P-loop NTPase
43	-3.6	6.62	2.6/4	1	1	58.9	ENSP00000378360	ENSP00000378360	TKFC:p, dihydroxyacetone kinase 2 homolog (S. cerevisiae) [Source: HGNC 24552] IPR004006 Dak1 IPR012734 Dhak ATP IPR004007 (x2) DhaL dom
44	-3.6	6.64	11/18	1	3	11.4	ENSP00000376669	ENSP00000376669	HIST2H4A:p, histone cluster 2, H4a [Source: HGNC 4794] IPR001951 Histone H4 IPR007125 Histone core D IPR004823 TAF TATA-bd
45	-3.4	6.14	1.4/2	1	1	122.2	ENSP00000261590	ENSP00000261590	DSG2:p, desmoglein 2 [Source:HGNC Symbol;Acc:HGNC:3049] IPR002126 (x17) Cadherin IPR000233 Cadherin, cytoplasmic domain IPR015919 (x5) Cadherin-like IPR009123 (x4) Desmoglein IPR009122 (x4) Desmosomal cadherin
46	-3.4	6.54	9.4/12	1	2	14.4	ENSP00000422078	ENSP00000422078	SUB1:p, SUB1 homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:HGNC:19985] IPR003173 Transcriptional coactivator p15 (PC4) IPR009044 ssDNA-binding transcriptional regulator
47	-3.4	6.02	5.1/8	1	1	24.5	sp CAS1_BOVIN	sp CAS1_BOVIN	Alpha-S1-casein; Bos d 8; Contains: Antioxidant peptide; Flags: Precursor;
48	-3.1	6.80	6.0/10	1	2	40.2	ENSP00000386101	ENSP00000386101	RPL3:p, ribosomal protein L3 [Source:HGNC Symbol;Acc:HGNC:10332] IPR000597 Ribosomal protein L3 IPR009000 Translation protein, beta-barrel domain
49	-3.0	6.74	10/20	1	1	14.5	ENSP00000264258	ENSP00000264258	RPL31:p, ribosomal protein L31 [Source:HGNC Symbol;Acc:HGNC:10334] IPR000054 Ribosomal protein L31e IPR020052 Ribosomal protein L31e, conserved site
50	-2.8	6.17	5.6/9	1	1	32.7	ENSP00000202773	ENSP00000202773	RPL6:p, ribosomal protein L6 [Source:HGNC Symbol;Acc:HGNC:10362] IPR000915 (x2) 60S ribosomal protein L6E IPR005568 Ribosomal protein L6, N-terminal IPR008991 Translation protein SH3-like domain
51	-2.4	6.05	3.6/5	1	1	42.8	ENSP00000304139	ENSP00000304139	RBML42:p, RNA binding motif protein, X-linked-like 2 [Source: HGNC 17886] IPR012604 RBMLCTR IPR000504 (x3) RRM dom IPR003954 RRM dom euk
52	-2.3	6.13	4.3/8	1	1	55.6	ENSP00000405574	ENSP00000405574	TBL1XR1:p, transducin (beta)-like 1X-linked receptor 1 [Source: HGNC 29529] IPR020472 (x3) G-protein beta WD-40 rep IPR006594 (x2) LSH dimerisation IPR013720 LSH dimerisation subgr IPR001680 (x21) WD40 repeat IPR017986 (x2) WD40 repeat dom
53	-2.1	5.02	4.2/6	1	1	49.9	ENSP00000404174	ENSP00000404174	no protein information available
54	-2.0	6.10	7.7/17	1	1	17.7	ENSP00000389103	ENSP00000389103	RPL23A:p, ribosomal protein L23a [Source:HGNC Symbol;Acc:HGNC:10317] IPR019985 Ribosomal protein L23 IPR012678 Ribosomal protein L23/L15e core domain IPR005633 Ribosomal protein L23/L25, N-terminal IPR001014 Ribosomal protein L23/L25, conserved site IPR013025 Ribosomal protein L25/L23
55	-1.8	6.16	1.4/2	1	1	127.0	ENSP00000373565	ENSP00000373565	FAM83Hp,
56	-1.8	6.48	2.4/4	1	1	86.9	ENSP00000363883	ENSP00000363883	CUL2:p,
57	-1.7	6.19	6.4/14	1	1	21.6	ENSP00000447001	ENSP00000447001	RPL18:p, ribosomal protein L18 [Source:HGNC Symbol;Acc:HGNC:10310] IPR021132 Ribosomal protein L18e, conserved site IPR021131 (x2) Ribosomal protein L18e/L15P
58	-1.6	5.61	0.2/0	1	1	564.2	ENSP00000355533	ENSP00000355533	RYR2:p, ryanodine receptor 2 (cardiac) [Source: HGNC 10484]

								IPR001870 B30.2/SPRY
								IPR000699 ($\times 2$) Ca-rel channel
								IPR008985 ($\times 3$) ConA-like lec gl sf
								IPR002048 EF hand dom
								IPR014821 Ins145 P3 rcpt
								IPR005821 Ion trans dom
								IPR016093 ($\times 11$) MIR motif
								IPR013662 RIH assoc-dom
								IPR013333 ($\times 9$) Ryan recept
								IPR003032 ($\times 4$) Ryanodine rcpt
								IPR009460 Ryanrecept TM4-6
								IPR003877 ($\times 3$) SPRY rcpt
								IPR018355 ($\times 3$) SPla/Ryanodine receptor subgr
59	-1.5	5.60	4.4/11	1	1	24.7	ENSP00000305938	ENSP00000305938 ✖ NEGR1.p,
60	-1.5	5.95	5.8/9	1	1	31.7	ENSP00000365076	ENSP00000365076 ✖ C6orf47.p,
61	-1.4	5.47	0.8/1	1	1	89.4	ENSP00000356452	ENSP00000356452 ✖ PRG4.p,
62	-1.4	5.76	1.1/1	1	1	163.7	ENSP00000361290	ENSP00000361290 ✖ COL4A6.p,
63	-1.3	5.99	8.5/11	1	1	51.7	ENSP00000253109	ENSP00000253109 ✖ ANGPT16.p,
64	-1.3	5.86	1.2/2	1	1	110.0	ENSP00000252996	ENSP00000252996 ✖ TAF4.p, TAF4 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 135kDa [Source:HGNC Symbol;Acc:HGNC:11537] IPR009072 Histone-fold IPR003894 ($\times 3$) TAFH/NH-R1 IPR007900 Transcription initiation factor TFIID component TAF4
8.5/14.4 #23 #18								

Appendix III

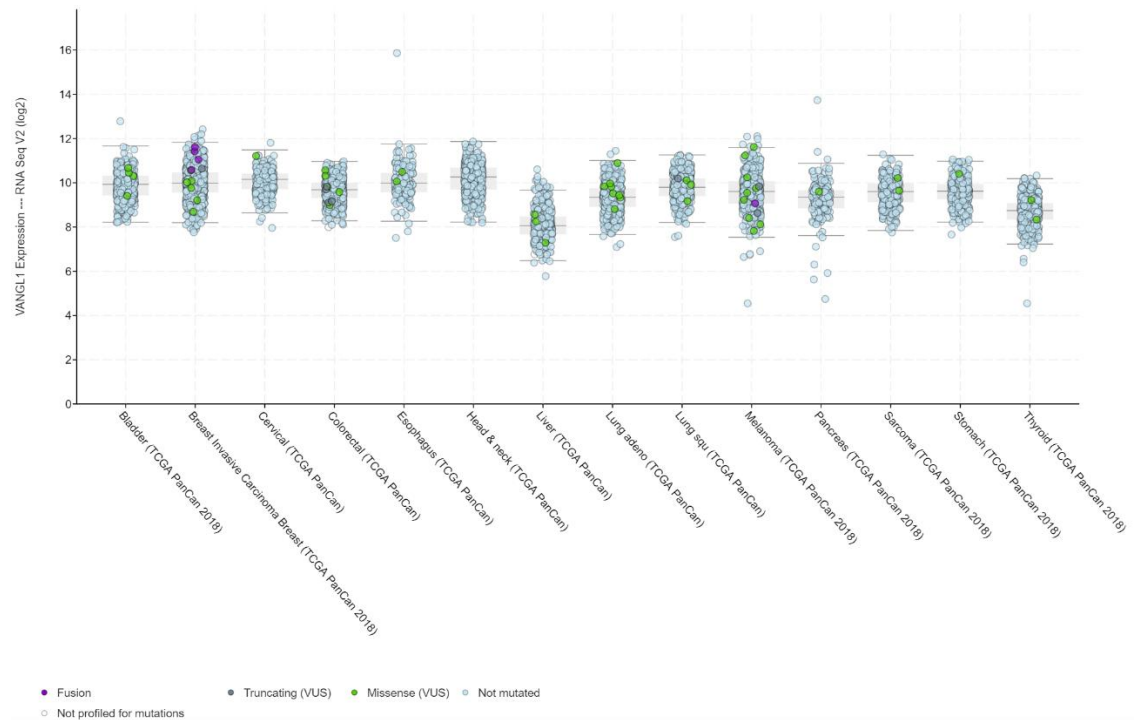


Figure 47. Vangl1 expression and mutation profile across several cancer data sets accessed via cBioPortal.

Bladder Urothelial Carcinoma (TCGA, PanCancer Atlas), Breast Invasive Carcinoma (TCGA, PanCancer Atlas), Cervical Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Colorectal Adenocarcinoma (TCGA, PanCancer Atlas), Esophageal Adenocarcinoma (TCGA, PanCancer Atlas), Head and Neck Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Liver Hepatocellular Carcinoma (TCGA, PanCancer Atlas), Lung Adenocarcinoma (TCGA, PanCancer Atlas), Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Pancreatic Adenocarcinoma (TCGA, PanCancer Atlas), Sarcoma (TCGA, PanCancer Atlas), Skin Cutaneous Melanoma (TCGA, PanCancer Atlas), Stomach Adenocarcinoma (TCGA, PanCancer Atlas), Thyroid Carcinoma (TCGA, PanCancer Atlas)

Indicated data sets were analyzed to display expression of Vangl1 analysis using cBioPortal on Dec 5, 2019 to view mutation profiles in the data sets. The data can be accessed at -

[https://www.cbioportal.org/results/expression?Action=Submit&RPPA_SCORE_THRESHOLD=2.0&Z_SCORE_THRESHOLD=2.0&cancer_study_list=cesc_tcga_pan_can_atlas_2018%2Cskcm_tcga_pan_can_atlas_2018%2Cluad_tcga_pan_can_atlas_2018%2Cnscl_tcga_broad_2016%2Cbica_tcga_pan_can_atlas_2018%2Ccoadread_tcga_pan_can_atlas_2018%2Cbrca_tcga_pan_can_atlas_2018%2Cesca_tcga_pan_can_atlas_2018%2Cstad_tcga_pan_can_atlas_2018%2Chnsc_tcga_pan_can_atlas_2018%2Clihc_tcga_pan_can_atlas_2018%2Clusc_tcga_pan_c an_atlas_2018%2Cpaad_tcga_pan_can_atlas_2018%2Csarc_tcga_pan_can_atlas_2018%2Ct hca_tcga_pan_can_atlas_2018&case_set_id=all&data_priority=0&gene_list=VANGL1&geneset_list=%20&tab_index=tab_visualize](https://www.cbioportal.org/results/expression?Action=Submit&RPPA_SCORE_THRESHOLD=2.0&Z_SCORE_THRESHOLD=2.0&cancer_study_list=cesc_tcga_pan_can_atlas_2018%2Cskcm_tcga_pan_can_atlas_2018%2Cluad_tcga_pan_can_atlas_2018%2Cnscl_tcga_broad_2016%2Cbica_tcga_pan_can_atlas_2018%2Ccoadread_tcga_pan_can_atlas_2018%2Cbrca_tcga_pan_can_atlas_2018%2Cesca_tcga_pan_can_atlas_2018%2Cstad_tcga_pan_can_atlas_2018%2Chnsc_tcga_pan_can_atlas_2018%2Clihc_tcga_pan_can_atlas_2018%2Clusc_tcga_pan_can_atlas_2018%2Cpaad_tcga_pan_can_atlas_2018%2Csarc_tcga_pan_can_atlas_2018%2Cthca_tcga_pan_can_atlas_2018&case_set_id=all&data_priority=0&gene_list=VANGL1&geneset_list=%20&tab_index=tab_visualize)

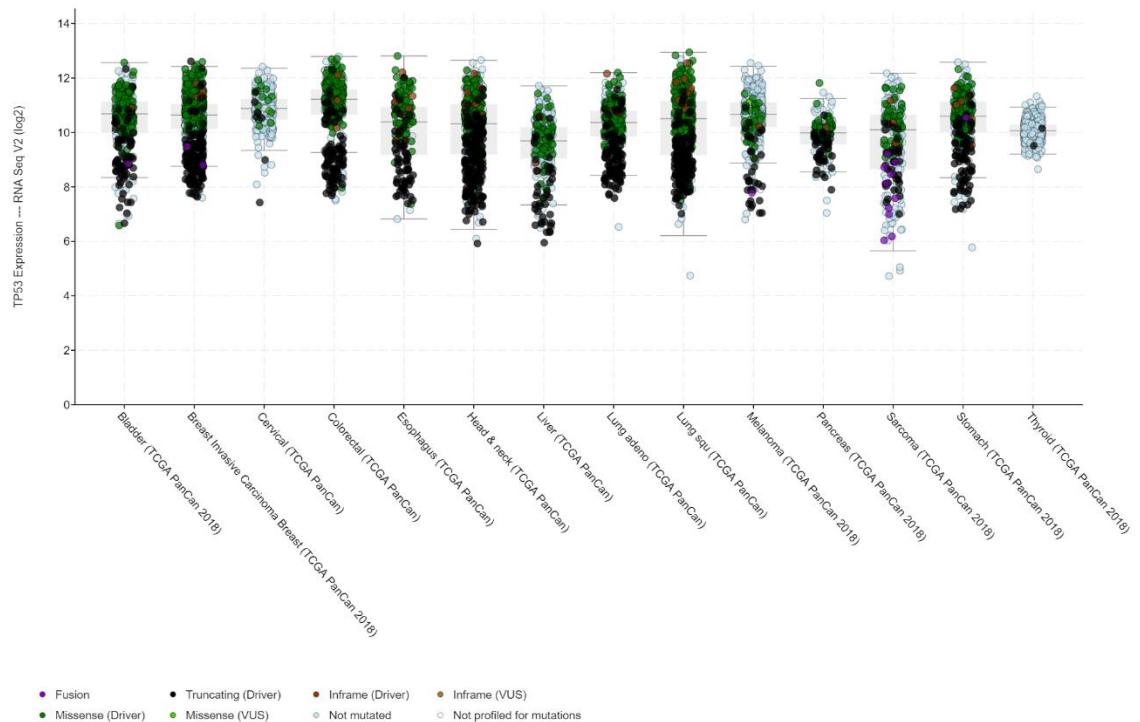


Figure 48. p53 expression and mutation profile across several cancer data sets accessed via cBioPortal.

Bladder Urothelial Carcinoma (TCGA, PanCancer Atlas), Breast Invasive Carcinoma (TCGA, PanCancer Atlas), Cervical Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Colorectal Adenocarcinoma (TCGA, PanCancer Atlas), Esophageal Adenocarcinoma (TCGA, PanCancer Atlas), Head and Neck Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Liver Hepatocellular Carcinoma (TCGA, PanCancer Atlas), Lung Adenocarcinoma (TCGA, PanCancer Atlas), Lung Squamous Cell Carcinoma (TCGA, PanCancer Atlas), Pancreatic Adenocarcinoma (TCGA, PanCancer Atlas), Sarcoma (TCGA, PanCancer Atlas), Skin Cutaneous Melanoma (TCGA, PanCancer Atlas), Stomach Adenocarcinoma (TCGA, PanCancer Atlas), Thyroid Carcinoma (TCGA, PanCancer Atlas)

Indicated data sets were analyzed to display expression of Vangl1 analysis using cBioPortal on Dec 5, 2019 to view mutation profiles in the data sets. The data can be accessed at –

https://www.cbioportal.org/results/expression?Action=Submit&RPPA_SCORE_THRESHOLD=2.0&Z_SCORE_THRESHOLD=2.0&cancer_study_list=cesc_tcga_pan_can_atlas_2018%2Cskcm_tcga_pan_can_atlas_2018%2Cluad_tcga_pan_can_atlas_2018%2Cnsclc_tcga_broad_2016%2Cblca_tcga_pan_can_atlas_2018%2Ccoadread_tcga_pan_can_atlas_2018%2Cbrca_tcga_pan_can_atlas_2018%2Cesca_tcga_pan_can_atlas_2018%2Cstad_tcga_pan_can_atlas_2018%2Chnsc_tcga_pan_can_atlas_2018%2Clihc_tcga_pan_can_atlas_2018%2Clusc_tcga_pan_c an_atlas_2018%2Cpaad_tcga_pan_can_atlas_2018%2Csarc_tcga_pan_can_atlas_2018%2Ct hca_tcga_pan_can_atlas_2018&case_set_id=all&clinicallist=CANCER_STUDY%2CNUM_SA MPLES_PER_PATIENT%2CPROFILED_IN_COPY_NUMBER_ALTERATION%2CPROFILED _IN_MUTATION_EXTENDED&data_priority=0&gene_list=VANGL1%252C%2520TP53%252C %2520RB1&geneset_list=%20&tab_index=tab_visualize

References

- Ackerman, P., Osheroff, N., 1989. Regulation of casein kinase II activity by epidermal growth factor in human A-431 carcinoma cells. *The Journal of biological chemistry* 264, 11958-11965.
- Ajiro, M., Tang, S., Doorbar, J., Zheng, Z.-M., 2016. Serine/Arginine-Rich Splicing Factor 3 and Heterogeneous Nuclear Ribonucleoprotein A1 Regulate Alternative RNA Splicing and Gene Expression of Human Papillomavirus 18 through Two Functionally Distinguishable *cis* Elements. *Journal of virology* 90, 9138-9152.
- Ajiro, M., Zheng, Z.-M., 2015. E6^ΔE7, a Novel Splice Isoform Protein of Human Papillomavirus 16, Stabilizes Viral E6 and E7 Oncoproteins via HSP90 and GRP78. *mBio* 6, e02068-02014.
- Akgul, B., Pfefferle, R., Marcuzzi, G.P., Zigrino, P., Krieg, T., Pfister, H., Mauch, C., 2006. Expression of matrix metalloproteinase (MMP)-2, MMP-9, MMP-13, and MT1-MMP in skin tumors of human papillomavirus type 8 transgenic mice. *Experimental dermatology* 15, 35-42.
- Alfandari, J., Shnitman Magal, S., Jackman, A., Schlegel, R., Gonen, P., Sherman, L., 1999. HPV16 E6 oncoprotein inhibits apoptosis induced during serum-calcium differentiation of foreskin human keratinocytes. *Virology* 257, 383-396.
- Allende, J.E., Allende, C.C., 1995. Protein kinases. 4. Protein kinase CK2: an enzyme with multiple substrates and a puzzling regulation. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 9, 313-323.
- Alonso, A., Pulido, R., 2016a. The extended human PTPome: a growing tyrosine phosphatase family. *The FEBS journal* 283, 1404-1429.
- Alonso, A., Pulido, R., 2016b. The extended human PTPome: a growing tyrosine phosphatase family. *The FEBS journal* 283, 2197-2201.
- Alonso, L.G., Garcia-Alai, M.M., Smal, C., Centeno, J.M., Iacono, R., Castano, E., Gualfetti, P., de Prat-Gay, G., 2004. The HPV16 E7 viral oncoprotein self-assembles into defined spherical oligomers. *Biochemistry* 43, 3310-3317.
- Alonso, L.G., Smal, C., Garcia-Alai, M.M., Chemes, L., Salame, M., de Prat-Gay, G., 2006. Chaperone holdase activity of human papillomavirus E7 oncoprotein. *Biochemistry* 45, 657-667.
- Anastas, J.N., Biechele, T.L., Robitaille, M., Muster, J., Allison, K.H., Angers, S., Moon, R.T., 2012. A protein complex of SCRIB, NOS1AP and VANG1 regulates cell polarity and migration, and is associated with breast cancer progression. *Oncogene* 31, 3696-3708.

- Angeline, M., Merle, E., Moroianu, J., 2003. The E7 oncoprotein of high-risk human papillomavirus type 16 enters the nucleus via a nonclassical Ran-dependent pathway. *Virology* 317, 13-23.
- Armstrong, D.J., Roman, A., 1995. Human papillomavirus type 6 E7 protein is a substrate in vitro of protein kinase C. *The Biochemical journal* 312 (Pt 3), 667-670.
- Asad, M., Wong, M.K., Tan, T.Z., Choolani, M., Low, J., Mori, S., Virshup, D., Thiery, J.P., Huang, R.Y., 2014. FZD7 drives in vitro aggressiveness in Stem-A subtype of ovarian cancer via regulation of non-canonical Wnt/PCP pathway. *Cell death & disease* 5, e1346.
- Assoian, R.K., 1997. Anchorage-dependent cell cycle progression. *The Journal of cell biology* 136, 1-4.
- Avvakumov, N., Torchia, J., Mymryk, J.S., 2003. Interaction of the HPV E7 proteins with the pCAF acetyltransferase. *Oncogene* 22, 3833-3841.
- Axelrod, J.D., 2001. Unipolar membrane association of Dishevelled mediates Frizzled planar cell polarity signaling. *Genes & development* 15, 1182-1187.
- Bae, S., Park, J., Kim, J.S., 2014. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. *Bioinformatics* 30, 1473-1475.
- Balbin, M., Fueyo, A., Tester, A.M., Pendas, A.M., Pitiot, A.S., Astudillo, A., Overall, C.M., Shapiro, S.D., Lopez-Otin, C., 2003. Loss of collagenase-2 confers increased skin tumor susceptibility to male mice. *Nature genetics* 35, 252-257.
- Banerjee, N.S., Wang, H.K., Broker, T.R., Chow, L.T., 2011. Human papillomavirus (HPV) E7 induces prolonged G2 following S phase reentry in differentiated human keratinocytes. *The Journal of biological chemistry* 286, 15473-15482.
- Banks, L., Edmonds, C., Vousden, K.H., 1990. Ability of the HPV16 E7 protein to bind RB and induce DNA synthesis is not sufficient for efficient transforming activity in NIH3T3 cells. *Oncogene* 5, 1383-1389.
- Barbosa, M.S., Edmonds, C., Fisher, C., Schiller, J.T., Lowy, D.R., Vousden, K.H., 1990. The region of the HPV E7 oncoprotein homologous to adenovirus E1a and Sv40 large T antigen contains separate domains for Rb binding and casein kinase II phosphorylation. *The EMBO journal* 9, 153-160.
- Barbosa, M.S., Lowy, D.R., Schiller, J.T., 1989. Papillomavirus polypeptides E6 and E7 are zinc-binding proteins. *Journal of virology* 63, 1404-1407.

- Barbosa, M.S., Vass, W.C., Lowy, D.R., Schiller, J.T., 1991. In vitro biological activities of the E6 and E7 genes vary among human papillomaviruses of different oncogenic potential. *Journal of virology* 65, 292-298.
- Barrow-Laing, L., Chen, W., Roman, A., 2010. Low- and high-risk human papillomavirus E7 proteins regulate p130 differently. *Virology* 400, 233-239.
- Bastock, R., Strutt, H., Strutt, D., 2003. Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. *Development* 130, 3007-3014.
- Bazzo Goulart, K.O., Guerra Godoy, A.E., Litvin, I.E., Firmbach Pasqualotto, F., 2017. Expression analysis of transglutaminase 2 in premalignant lesions of the cervix. *Applied Cancer Research* 37, 27.
- Bedell, M.A., Jones, K.H., Grossman, S.R., Laimins, L.A., 1989. Identification of human papillomavirus type 18 transforming genes in immortalized and primary cells. *Journal of virology* 63, 1247-1255.
- Belle, L., Ali, N., Lonic, A., Li, X., Paltridge, J.L., Roslan, S., Herrmann, D., Conway, J.R., Gehling, F.K., Bert, A.G., Crocker, L.A., Tsykin, A., Farshid, G., Goodall, G.J., Timpson, P., Daly, R.J., Khew-Goodall, Y., 2015. The tyrosine phosphatase PTPN14 (Pez) inhibits metastasis by altering protein trafficking. *Science signaling* 8, ra18.
- Belotti, E., Puvirajesinghe, T.M., Audebert, S., Baudalet, E., Camoin, L., Pierres, M., Lasvaux, L., Ferracci, G., Montcouquiol, M., Borg, J.P., 2012. Molecular characterisation of endogenous Vangl2/Vangl1 heteromeric protein complexes. *PloS one* 7, e46213.
- Ben-Saadon, R., Fajerman, I., Ziv, T., Hellman, U., Schwartz, A.L., Ciechanover, A., 2004. The tumor suppressor protein p16(INK4a) and the human papillomavirus oncoprotein-58 E7 are naturally occurring lysine-less proteins that are degraded by the ubiquitin system. Direct evidence for ubiquitination at the N-terminal residue. *The Journal of biological chemistry* 279, 41414-41421.
- Berezutskaya, E., Bagchi, S., 1997. The human papillomavirus E7 oncoprotein functionally interacts with the S4 subunit of the 26 S proteasome. *The Journal of biological chemistry* 272, 30135-30140.
- Bergant Marusic, M., Ozbun, M.A., Campos, S.K., Myers, M.P., Banks, L., 2012. Human papillomavirus L2 facilitates viral escape from late endosomes via sorting nexin 17. *Traffic* 13, 455-467.
- Bibby, A.C., Litchfield, D.W., 2005. The multiple personalities of the regulatory subunit of protein kinase CK2: CK2 dependent and CK2 independent roles reveal a secret identity for CK2beta. *International journal of biological sciences* 1, 67-79.

- Bischof, O., Nacerddine, K., Dejean, A., 2005. Human papillomavirus oncoprotein E7 targets the promyelocytic leukemia protein and circumvents cellular senescence via the Rb and p53 tumor suppressor pathways. *Molecular and cellular biology* 25, 1013-1024.
- Bodily, J.M., Mehta, K.P., Cruz, L., Meyers, C., Laimins, L.A., 2011a. The E7 open reading frame acts in cis and in trans to mediate differentiation-dependent activities in the human papillomavirus type 16 life cycle. *Journal of virology* 85, 8852-8862.
- Bodily, J.M., Mehta, K.P., Laimins, L.A., 2011b. Human papillomavirus E7 enhances hypoxia-inducible factor 1-mediated transcription by inhibiting binding of histone deacetylases. *Cancer research* 71, 1187-1195.
- Bonilla-Delgado, J., Bulut, G., Liu, X., Cortes-Malagon, E.M., Schlegel, R., Flores-Maldonado, C., Contreras, R.G., Chung, S.H., Lambert, P.F., Uren, A., Gariglio, P., 2012. The E6 oncoprotein from HPV16 enhances the canonical Wnt/beta-catenin pathway in skin epidermis in vivo. *Molecular cancer research : MCR* 10, 250-258.
- Bonilla, X., Parmentier, L., King, B., Bezrukov, F., Kaya, G., Zoete, V., Seplyarskiy, V.B., Sharpe, H.J., McKee, T., Letourneau, A., Ribaux, P.G., Popadin, K., Basset-Seguin, N., Ben Chaabene, R., Santoni, F.A., Andrianova, M.A., Guipponi, M., Garieri, M., Verdan, C., Grosdemange, K., Sumara, O., Eilers, M., Aifantis, I., Michielin, O., de Sauvage, F.J., Antonarakis, S.E., Nikolaev, S.I., 2016. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nature genetics* 48, 398-406.
- Boon, S.S., Banks, L., 2013. High-risk human papillomavirus E6 oncoproteins interact with 14-3-3zeta in a PDZ binding motif-dependent manner. *Journal of virology* 87, 1586-1595.
- Boon, S.S., Tomaic, V., Thomas, M., Roberts, S., Banks, L., 2015. Cancer-causing human papillomavirus E6 proteins display major differences in the phospho-regulation of their PDZ interactions. *Journal of virology* 89, 1579-1586.
- Bosch, F.X., Lorincz, A., Munoz, N., Meijer, C.J., Shah, K.V., 2002. The causal relation between human papillomavirus and cervical cancer. *Journal of clinical pathology* 55, 244-265.
- Boyer, S.N., Wazer, D.E., Band, V., 1996. E7 protein of human papilloma virus-16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer research* 56, 4620-4624.
- Bracken, A.P., Helin, K., 2009. Polycomb group proteins: navigators of lineage pathways led astray in cancer. *Nature reviews. Cancer* 9, 773-784.
- Branca, M., Giorgi, C., Ciotti, M., Santini, D., Di Bonito, L., Costa, S., Benedetto, A., Bonifacio, D., Di Bonito, P., Paba, P., Accardi, L., Mariani, L., Ruutu,

- M., Syrjanen, S., Favalli, C., Syrjanen, K., 2006. Upregulation of telomerase (hTERT) is related to the grade of cervical intraepithelial neoplasia, but is not an independent predictor of high-risk human papillomavirus, virus persistence, or disease outcome in cervical cancer. *Diagnostic cytopathology* 34, 739-748.
- Brehm, A., Nielsen, S.J., Miska, E.A., McCance, D.J., Reid, J.L., Bannister, A.J., Kouzarides, T., 1999. The E7 oncoprotein associates with Mi2 and histone deacetylase activity to promote cell growth. *The EMBO journal* 18, 2449-2458.
- Brew, K., Nagase, H., 2010. The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. *Biochimica et biophysica acta* 1803, 55-71.
- Brimer, N., Drews, C.M., Vande Pol, S.B., 2017. Association of papillomavirus E6 proteins with either MAML1 or E6AP clusters E6 proteins by structure, function, and evolutionary relatedness. *PLoS pathogens* 13, e1006781.
- Brimer, N., Lyons, C., Vande Pol, S.B., 2007. Association of E6AP (UBE3A) with human papillomavirus type 11 E6 protein. *Virology* 358, 303-310.
- Brimer, N., Lyons, C., Wallberg, A.E., Vande Pol, S.B., 2012. Cutaneous papillomavirus E6 oncoproteins associate with MAML1 to repress transactivation and NOTCH signaling. *Oncogene* 31, 4639-4646.
- Bruni L, B.-R.L., Albero G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX, de Sanjosé S, ICO Information Centre on HPV and Cancer (HPV Information Centre), 2017. Human Papillomavirus and Related Diseases Report.
- Buck, C.B., Cheng, N., Thompson, C.D., Lowy, D.R., Steven, A.C., Schiller, J.T., Trus, B.L., 2008. Arrangement of L2 within the papillomavirus capsid. *Journal of virology* 82, 5190-5197.
- Buck, C.B., Thompson, C.D., Pang, Y.Y., Lowy, D.R., Schiller, J.T., 2005. Maturation of papillomavirus capsids. *Journal of virology* 79, 2839-2846.
- Burgers, W.A., Blanchon, L., Pradhan, S., de Launoit, Y., Kouzarides, T., Fuks, F., 2007. Viral oncoproteins target the DNA methyltransferases. *Oncogene* 26, 1650-1655.
- Butz, K., Ristriani, T., Hengstermann, A., Denk, C., Scheffner, M., Hoppe-Seyler, F., 2003. siRNA targeting of the viral E6 oncogene efficiently kills human papillomavirus-positive cancer cells. *Oncogene* 22, 5938-5945.
- Calcada, E.O., Felli, I.C., Hosek, T., Pierattelli, R., 2013. The heterogeneous structural behavior of E7 from HPV16 revealed by NMR spectroscopy. *Chembiochem : a European journal of chemical biology* 14, 1876-1882.
- Cardone, L., Carlucci, A., Affaitati, A., Livigni, A., DeCristofaro, T., Garbi, C., Varrone, S., Ullrich, A., Gottesman, M.E., Avvedimento, E.V., Feliciello,

- A., 2004. Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. *Molecular and cellular biology* 24, 4613-4626.
- Carlucci, A., Gedressi, C., Lignitto, L., Nezi, L., Villa-Moruzzi, E., Avvedimento, E.V., Gottesman, M., Garbi, C., Feliciello, A., 2008. Protein-tyrosine phosphatase PTPD1 regulates focal adhesion kinase autophosphorylation and cell migration. *The Journal of biological chemistry* 283, 10919-10929.
- Carlucci, A., Porpora, M., Garbi, C., Galgani, M., Santoriello, M., Mascolo, M., di Lorenzo, D., Altieri, V., Quarto, M., Terracciano, L., Gottesman, M.E., Insabato, L., Feliciello, A., 2010. PTPD1 supports receptor stability and mitogenic signaling in bladder cancer cells. *The Journal of biological chemistry* 285, 39260-39270.
- Carvajal-Gonzalez, J.M., Balmer, S., Mendoza, M., Dussert, A., Collu, G., Roman, A.C., Weber, U., Ciruna, B., Mlodzik, M., 2015. The clathrin adaptor AP-1 complex and Arf1 regulate planar cell polarity in vivo. *Nature communications* 6, 6751.
- Cetin, G.O., Toylu, A., Atabey, N., Sercan, Z., Sakizli, M., 2015. Downregulation of VANGL1 inhibits cellular invasion rather than cell motility in hepatocellular carcinoma cells without stimulation. *Genetic testing and molecular biomarkers* 19, 283-287.
- Chand, V., John, R., Jaiswal, N., Johar, S.S., Nag, A., 2014. High-risk HPV16E6 stimulates hADA3 degradation by enhancing its SUMOylation. *Carcinogenesis* 35, 1830-1839.
- Chaussepied, M., Ginsberg, D., 2004. Transcriptional regulation of AKT activation by E2F. *Molecular cell* 16, 831-837.
- Chemes, L.B., Sanchez, I.E., Smal, C., de Prat-Gay, G., 2010. Targeting mechanism of the retinoblastoma tumor suppressor by a prototypical viral oncoprotein. Structural modularity, intrinsic disorder and phosphorylation of human papillomavirus E7. *The FEBS journal* 277, 973-988.
- Chen, H., Zhang, Q., Qiao, L., Fan, X., Zhang, W., Zhao, W., Chen, J.J., 2017. Cdc6 contributes to abrogating the G1 checkpoint under hypoxic conditions in HPV E7 expressing cells. *Scientific reports* 7, 2927.
- Chen, J.J., Hong, Y., Rustamzadeh, E., Baleja, J.D., Androphy, E.J., 1998. Identification of an alpha helical motif sufficient for association with papillomavirus E6. *The Journal of biological chemistry* 273, 13537-13544.
- Chen, W.S., Antic, D., Matis, M., Logan, C.Y., Povelones, M., Anderson, G.A., Nusse, R., Axelrod, J.D., 2008. Asymmetric homotypic interactions of the atypical cadherin flamingo mediate intercellular polarity signaling. *Cell* 133, 1093-1105.

- Chien, W.M., Parker, J.N., Schmidt-Grimminger, D.C., Broker, T.R., Chow, L.T., 2000. Casein kinase II phosphorylation of the human papillomavirus-18 E7 protein is critical for promoting S-phase entry. *Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research* 11, 425-435.
- Chin, Y.R., Toker, A., 2009. Function of Akt/PKB signaling to cell motility, invasion and the tumor stroma in cancer. *Cellular signalling* 21, 470-476.
- Cho, B., Pierre-Louis, G., Sagner, A., Eaton, S., Axelrod, J.D., 2015. Clustering and negative feedback by endocytosis in planar cell polarity signaling is modulated by ubiquitylation of prickle. *PLoS genetics* 11, e1005259.
- Cho, S.W., Kim, S., Kim, Y., Kweon, J., Kim, H.S., Bae, S., Kim, J.S., 2014. Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome research* 24, 132-141.
- Chua, M.M.J., Lee, M., Dominguez, I., 2017a. Cancer-type dependent expression of CK2 transcripts. *PloS one* 12, e0188854.
- Chua, M.M.J., Ortega, C.E., Sheikh, A., Lee, M., Abdul-Rassoul, H., Hartshorn, K.L., Dominguez, I., 2017b. CK2 in Cancer: Cellular and Biochemical Mechanisms and Potential Therapeutic Target. *Pharmaceuticals* 10, 18.
- Clemens, K.E., Brent, R., Gyuris, J., Munger, K., 1995. Dimerization of the human papillomavirus E7 oncoprotein in vivo. *Virology* 214, 289-293.
- Clements, A., Johnston, K., Mazzearelli, J.M., Ricciardi, R.P., Marmorstein, R., 2000. Oligomerization properties of the viral oncoproteins adenovirus E1A and human papillomavirus E7 and their complexes with the retinoblastoma protein. *Biochemistry* 39, 16033-16045.
- Cogliano, V.J., Baan, R., Straif, K., Grosse, Y., Lauby-Secretan, B., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L., Wild, C.P., 2011. Preventable exposures associated with human cancers. *Journal of the National Cancer Institute* 103, 1827-1839.
- Conrad, M., Bubb, V.J., Schlegel, R., 1993. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. *Journal of virology* 67, 6170-6178.
- Contreras-Paredes, A., De la Cruz-Hernandez, E., Martinez-Ramirez, I., Duenas-Gonzalez, A., Lizano, M., 2009. E6 variants of human papillomavirus 18 differentially modulate the protein kinase B/phosphatidylinositol 3-kinase (akt/PI3K) signaling pathway. *Virology* 383, 78-85.
- Courbard, J.R., Djiane, A., Wu, J., Mlodzik, M., 2009. The apical/basal-polarity determinant Scribble cooperates with the PCP core factor Stbm/Vang and functions as one of its effectors. *Developmental biology* 333, 67-77.

- Culp, T.D., Budgeon, L.R., Marinkovich, M.P., Meneguzzi, G., Christensen, N.D., 2006. Keratinocyte-secreted laminin 5 can function as a transient receptor for human papillomaviruses by binding virions and transferring them to adjacent cells. *Journal of virology* 80, 8940-8950.
- Cutts, F.T., Franceschi, S., Goldie, S., Castellsague, X., de Sanjose, S., Garnett, G., Edmunds, W.J., Claeys, P., Goldenthal, K.L., Harper, D.M., Markowitz, L., 2007. Human papillomavirus and HPV vaccines: a review. *Bulletin of the World Health Organization* 85, 719-726.
- Daulat, A.M., Bertucci, F., Audebert, S., Serge, A., Finetti, P., Josselin, E., Castellano, R., Birnbaum, D., Angers, S., Borg, J.P., 2016. PRICKLE1 Contributes to Cancer Cell Dissemination through Its Interaction with mTORC2. *Developmental cell* 37, 311-325.
- Daulat, A.M., Borg, J.P., 2016. When mTORC2-AKT signaling meets cell polarity. *Cell cycle* 15, 3003-3004.
- Daulat, A.M., Luu, O., Sing, A., Zhang, L., Wrana, J.L., McNeill, H., Winklbauer, R., Angers, S., 2012. Mink1 regulates beta-catenin-independent Wnt signaling via Prickle phosphorylation. *Molecular and cellular biology* 32, 173-185.
- Day, P.M., Roden, R.B., Lowy, D.R., Schiller, J.T., 1998. The papillomavirus minor capsid protein, L2, induces localization of the major capsid protein, L1, and the viral transcription/replication protein, E2, to PML oncogenic domains. *Journal of virology* 72, 142-150.
- de Freitas, A.C., de Oliveira, T.H.A., Barros, M.R., Jr., Venuti, A., 2017. hrHPV E5 oncoprotein: immune evasion and related immunotherapies. *Journal of experimental & clinical cancer research : CR* 36, 71.
- de Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D., Plummer, M., 2012. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *The Lancet. Oncology* 13, 607-615.
- de Martel, C., Plummer, M., Vignat, J., Franceschi, S., 2017. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *International journal of cancer. Journal international du cancer* 141, 664-670.
- DeFilippis, R.A., Goodwin, E.C., Wu, L., DiMaio, D., 2003. Endogenous human papillomavirus E6 and E7 proteins differentially regulate proliferation, senescence, and apoptosis in HeLa cervical carcinoma cells. *Journal of virology* 77, 1551-1563.
- Del Nonno, F., Pisani, G., Visca, P., Signore, F., Grillo, L.R., Baiocchi, A., Garbuglia, A.R., Sepe, S., Piacentini, M., Falasca, L., 2011. Role and predictive strength of transglutaminase type 2 expression in premalignant lesions of the cervix. *Modern Pathology* 24, 855.
- DeMasi, J., Chao, M.C., Kumar, A.S., Howley, P.M., 2007. Bovine papillomavirus E7 oncoprotein inhibits anoikis. *Journal of virology* 81, 9419-9425.

- DeMasi, J., Huh, K.W., Nakatani, Y., Munger, K., Howley, P.M., 2005. Bovine papillomavirus E7 transformation function correlates with cellular p600 protein binding. *Proceedings of the National Academy of Sciences of the United States of America* 102, 11486-11491.
- Demers, G.W., Foster, S.A., Halbert, C.L., Galloway, D.A., 1994. Growth arrest by induction of p53 in DNA damaged keratinocytes is bypassed by human papillomavirus 16 E7. *Proceedings of the National Academy of Sciences of the United States of America* 91, 4382-4386.
- Devenport, D., 2014. The cell biology of planar cell polarity. *The Journal of cell biology* 207, 171-179.
- Devenport, D., Fuchs, E., 2008. Planar polarization in embryonic epidermis orchestrates global asymmetric morphogenesis of hair follicles. *Nature cell biology* 10, 1257-1268.
- Dick, F.A., Dyson, N.J., 2002. Three regions of the pRB pocket domain affect its inactivation by human papillomavirus E7 proteins. *Journal of virology* 76, 6224-6234.
- DiPaolo, J.A., Woodworth, C.D., Popescu, N.C., Notario, V., Doniger, J., 1989. Induction of human cervical squamous cell carcinoma by sequential transfection with human papillomavirus 16 DNA and viral Harvey ras. *Oncogene* 4, 395-399.
- Dobrosotskaya, I., Guy, R.K., James, G.L., 1997. MAGI-1, a Membrane-associated Guanylate Kinase with a Unique Arrangement of Protein-Protein Interaction Domains. *Journal of Biological Chemistry* 272, 31589-31597.
- Doorbar, J., 2005. The papillomavirus life cycle. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 32 Suppl 1, S7-15.
- Doorbar, J., 2006. Molecular biology of human papillomavirus infection and cervical cancer. *Clinical science* 110, 525-541.
- Doorbar, J., 2013. The E4 protein; structure, function and patterns of expression. *Virology* 445, 80-98.
- Doorbar, J., Egawa, N., Griffin, H., Kranjec, C., Murakami, I., 2015. Human papillomavirus molecular biology and disease association. *Reviews in medical virology* 25 Suppl 1, 2-23.
- Doorbar, J., Parton, A., Hartley, K., Banks, L., Crook, T., Stanley, M., Crawford, L., 1990. Detection of novel splicing patterns in a HPV16-containing keratinocyte cell line. *Virology* 178, 254-262.
- Doorbar, J., Quint, W., Banks, L., Bravo, I.G., Stoler, M., Broker, T.R., Stanley, M.A., 2012. The biology and life-cycle of human papillomaviruses. *Vaccine* 30 Suppl 5, F55-70.

- Dorner, C., Ciossek, T., Muller, S., Moller, P.H., Ullrich, A., Lammers, R., 1998. Characterization of KIF1C, a new kinesin-like protein involved in vesicle transport from the Golgi apparatus to the endoplasmic reticulum. *The Journal of biological chemistry* 273, 20267-20275.
- Dowhanick, J.J., McBride, A.A., Howley, P.M., 1995. Suppression of cellular proliferation by the papillomavirus E2 protein. *Journal of virology* 69, 7791-7799.
- Duensing, S., Lee, L.Y., Duensing, A., Basile, J., Piboonniyom, S., Gonzalez, S., Crum, C.P., Munger, K., 2000. The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proceedings of the National Academy of Sciences of the United States of America* 97, 10002-10007.
- Duensing, S., Munger, K., 2003. Human papillomavirus type 16 E7 oncoprotein can induce abnormal centrosome duplication through a mechanism independent of inactivation of retinoblastoma protein family members. *Journal of virology* 77, 12331-12335.
- Durst, M., Gallahan, D., Jay, G., Rhim, J.S., 1989. Glucocorticoid-enhanced neoplastic transformation of human keratinocytes by human papillomavirus type 16 and an activated ras oncogene. *Virology* 173, 767-771.
- Dyson, N., 1998. The regulation of E2F by pRB-family proteins. *Genes & development* 12, 2245-2262.
- Dyson, N., Guida, P., Munger, K., Harlow, E., 1992. Homologous sequences in adenovirus E1A and human papillomavirus E7 proteins mediate interaction with the same set of cellular proteins. *Journal of virology* 66, 6893-6902.
- Dyson, N., Howley, P.M., Munger, K., Harlow, E., 1989. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243, 934-937.
- el-Deiry, W.S., Tokino, T., Velculescu, V.E., Levy, D.B., Parsons, R., Trent, J.M., Lin, D., Mercer, W.E., Kinzler, K.W., Vogelstein, B., 1993. WAF1, a potential mediator of p53 tumor suppression. *Cell* 75, 817-825.
- Evan, G.I., Vousden, K.H., 2001. Proliferation, cell cycle and apoptosis in cancer. *Nature* 411, 342-348.
- Evander, M., Frazer, I.H., Payne, E., Qi, Y.M., Hengst, K., McMillan, N.A., 1997. Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. *Journal of virology* 71, 2449-2456.
- Fan, X., Zhou, Y., Chen, J.J., 2016. Role of Cdc6 in re-replication in cells expressing human papillomavirus E7 oncogene. *Carcinogenesis* 37, 799-809.

- Fang, F., Orend, G., Watanabe, N., Hunter, T., Ruoslahti, E., 1996. Dependence of cyclin E-CDK2 kinase activity on cell anchorage. *Science* 271, 499-502.
- Figge, J., Smith, T.F., 1988. Cell-division sequence motif. *Nature* 334, 109.
- Filippova, M., Johnson, M.M., Bautista, M., Filippov, V., Fodor, N., Tungteakkhun, S.S., Williams, K., Duerksen-Hughes, P.J., 2007. The Large and Small Isoforms of Human Papillomavirus Type 16 E6 Bind to and Differentially Affect Procaspase 8 Stability and Activity. *Journal of virology* 81, 4116-4129.
- Filippova, M., Parkhurst, L., Duerksen-Hughes, P.J., 2004. The Human Papillomavirus 16 E6 Protein Binds to Fas-associated Death Domain and Protects Cells from Fas-triggered Apoptosis. *Journal of Biological Chemistry* 279, 25729-25744.
- Finnen, R.L., Erickson, K.D., Chen, X.S., Garcea, R.L., 2003. Interactions between papillomavirus L1 and L2 capsid proteins. *Journal of virology* 77, 4818-4826.
- Firpo, E.J., Koff, A., Solomon, M.J., Roberts, J.M., 1994. Inactivation of a Cdk2 inhibitor during interleukin 2-induced proliferation of human T lymphocytes. *Molecular and cellular biology* 14, 4889-4901.
- Firzlaff, J.M., Galloway, D.A., Eisenman, R.N., Lüscher, B., 1989. The E7 protein of human papillomavirus type 16 is phosphorylated by casein kinase II. *The New biologist* 1, 44-53.
- Firzlaff, J.M., Lüscher, B., Eisenman, R.N., 1991. Negative charge at the casein kinase II phosphorylation site is important for transformation but not for Rb protein binding by the E7 protein of human papillomavirus type 16. *Proceedings of the National Academy of Sciences of the United States of America* 88, 5187-5191.
- Folgueras, A.R., Pendas, A.M., Sanchez, L.M., Lopez-Otin, C., 2004. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *The International journal of developmental biology* 48, 411-424.
- Frisch, S.M., Screaton, R.A., 2001. Anoikis mechanisms. *Current opinion in cell biology* 13, 555-562.
- Frolov, M.V., Dyson, N.J., 2004. Molecular mechanisms of E2F-dependent activation and pRB-mediated repression. *Journal of cell science* 117, 2173-2181.
- Funk, J.O., Waga, S., Harry, J.B., Espling, E., Stillman, B., Galloway, D.A., 1997. Inhibition of CDK activity and PCNA-dependent DNA replication by p21 is blocked by interaction with the HPV-16 E7 oncoprotein. *Genes & development* 11, 2090-2100.

- Gage, J.R., Meyers, C., Wettstein, F.O., 1990. The E7 proteins of the nononcogenic human papillomavirus type 6b (HPV-6b) and of the oncogenic HPV-16 differ in retinoblastoma protein binding and other properties. *Journal of virology* 64, 723-730.
- Ganti, K., Massimi, P., Manzo-Merino, J., Tomaic, V., Pim, D., Playford, M.P., Lizano, M., Roberts, S., Kranjec, C., Doorbar, J., Banks, L., 2016. Interaction of the Human Papillomavirus E6 Oncoprotein with Sorting Nexin 27 Modulates Endocytic Cargo Transport Pathways. *PLoS pathogens* 12, e1005854.
- Garcia-Alai, M.M., Alonso, L.G., de Prat-Gay, G., 2007. The N-terminal module of HPV16 E7 is an intrinsically disordered domain that confers conformational and recognition plasticity to the oncoprotein. *Biochemistry* 46, 10405-10412.
- Gardiol, D., Kuhne, C., Glaunsinger, B., Lee, S.S., Javier, R., Banks, L., 1999. Oncogenic human papillomavirus E6 proteins target the discs large tumour suppressor for proteasome-mediated degradation. *Oncogene* 18, 5487-5496.
- Genovese, N.J., Banerjee, N.S., Broker, T.R., Chow, L.T., 2008. Casein kinase II motif-dependent phosphorylation of human papillomavirus E7 protein promotes p130 degradation and S-phase induction in differentiated human keratinocytes. *Journal of virology* 82, 4862-4873.
- Genovese, N.J., Broker, T.R., Chow, L.T., 2011. Nonconserved lysine residues attenuate the biological function of the low-risk human papillomavirus E7 protein. *Journal of virology* 85, 5546-5554.
- Gewin, L., Myers, H., Kiyono, T., Galloway, D.A., 2004. Identification of a novel telomerase repressor that interacts with the human papillomavirus type-16 E6/E6-AP complex. *Genes & development* 18, 2269-2282.
- Giarre, M., Caldeira, S., Malanchi, I., Ciccolini, F., Leao, M.J., Tommasino, M., 2001. Induction of pRb degradation by the human papillomavirus type 16 E7 protein is essential to efficiently overcome p16INK4a-imposed G1 cell cycle Arrest. *Journal of virology* 75, 4705-4712.
- Gilet, A., Zou, F., Boumenir, M., Fripiat, J.P., Thornton, S.N., Lacolley, P., Ropars, A., 2015. Aldosterone up-regulates MMP-9 and MMP-9/NGAL expression in human neutrophils through p38, ERK1/2 and PI3K pathways. *Experimental cell research* 331, 152-163.
- Giroglou, T., Florin, L., Schafer, F., Streeck, R.E., Sapp, M., 2001. Human papillomavirus infection requires cell surface heparan sulfate. *Journal of virology* 75, 1565-1570.
- Glaunsinger, B.A., Lee, S.S., Thomas, M., Banks, L., Javier, R., 2000. Interactions of the PDZ-protein MAGI-1 with adenovirus E4-ORF1 and high-risk papillomavirus E6 oncoproteins. *Oncogene* 19, 5270-5280.

GLOBOCAN, 2012.

- Gonzalez, S.L., Stremlau, M., He, X., Basile, J.R., Munger, K., 2001. Degradation of the retinoblastoma tumor suppressor by the human papillomavirus type 16 E7 oncoprotein is important for functional inactivation and is separable from proteasomal degradation of E7. *Journal of virology* 75, 7583-7591.
- Greenfield, I., Nickerson, J., Penman, S., Stanley, M., 1991. Human papillomavirus 16 E7 protein is associated with the nuclear matrix. *Proceedings of the National Academy of Sciences of the United States of America* 88, 11217-11221.
- Grossman, S.R., Laimins, L.A., 1989. E6 protein of human papillomavirus type 18 binds zinc. *Oncogene* 4, 1089-1093.
- Guccione, E., Massimi, P., Bernat, A., Banks, L., 2002. Comparative analysis of the intracellular location of the high- and low-risk human papillomavirus oncoproteins. *Virology* 293, 20-25.
- Guirouilh-Barbat, J., Huck, S., Bertrand, P., Pirzio, L., Desmaze, C., Sabatier, L., Lopez, B.S., 2004. Impact of the KU80 pathway on NHEJ-induced genome rearrangements in mammalian cells. *Molecular cell* 14, 611-623.
- Gulliver, G.A., Herber, R.L., Liem, A., Lambert, P.F., 1997. Both conserved region 1 (CR1) and CR2 of the human papillomavirus type 16 E7 oncogene are required for induction of epidermal hyperplasia and tumor formation in transgenic mice. *Journal of virology* 71, 5905-5914.
- Halbert, C.L., Demers, G.W., Galloway, D.A., 1992. The E6 and E7 genes of human papillomavirus type 6 have weak immortalizing activity in human epithelial cells. *Journal of virology* 66, 2125-2134.
- Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. *Cell* 100, 57-70.
- Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: the next generation. *Cell* 144, 646-674.
- Hanahan, D., Weinberg, R.A., 2016. Biological Hallmarks of Cancer, *Holland-Frei Cancer Medicine*, pp. 1-10.
- Harden, M.E., Prasad, N., Griffiths, A., Munger, K., 2017. Modulation of microRNA-mRNA Target Pairs by Human Papillomavirus 16 Oncoproteins. *mBio* 8.
- Hatakeyama, J., Wald, J.H., Printsev, I., Ho, H.Y., Carraway, K.L., 3rd, 2014. Vangl1 and Vangl2: planar cell polarity components with a developing role in cancer. *Endocrine-related cancer* 21, R345-356.
- Hatterschide, J., Bohidar, A.E., Grace, M., Nulton, T.J., Kim, H.W., Windle, B., Morgan, I.M., Munger, K., White, E.A., 2019. PTPN14 degradation by high-risk human papillomavirus E7 limits keratinocyte differentiation and

contributes to HPV-mediated oncogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 116, 7033-7042.

- Haukioja, A., Tervahartiala, T., Sorsa, T., Syrjanen, S., 2017. Persistent Oral Human Papillomavirus (HPV) Infection is Associated with Low Salivary Levels of Matrix Metalloproteinase 8 (MMP-8). *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology* 97, 4-9.
- Hawley-Nelson, P., Vousden, K.H., Hubbert, N.L., Lowy, D.R., Schiller, J.T., 1989. HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. *The EMBO journal* 8, 3905-3910.
- He, W., Staples, D., Smith, C., Fisher, C., 2003. Direct activation of cyclin-dependent kinase 2 by human papillomavirus E7. *Journal of virology* 77, 10566-10574.
- Heck, D.V., Yee, C.L., Howley, P.M., Munger, K., 1992. Efficiency of binding the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. *Proceedings of the National Academy of Sciences of the United States of America* 89, 4442-4446.
- Helt, A.M., Funk, J.O., Galloway, D.A., 2002. Inactivation of both the retinoblastoma tumor suppressor and p21 by the human papillomavirus type 16 E7 oncoprotein is necessary to inhibit cell cycle arrest in human epithelial cells. *Journal of virology* 76, 10559-10568.
- Helt, A.M., Galloway, D.A., 2001. Destabilization of the retinoblastoma tumor suppressor by human papillomavirus type 16 E7 is not sufficient to overcome cell cycle arrest in human keratinocytes. *Journal of virology* 75, 6737-6747.
- Hengstermann, A., D'Silva M, A., Kuballa, P., Butz, K., Hoppe-Seyler, F., Scheffner, M., 2005. Growth suppression induced by downregulation of E6-AP expression in human papillomavirus-positive cancer cell lines depends on p53. *Journal of virology* 79, 9296-9300.
- Holland, D., Hoppe-Seyler, K., Schuller, B., Lohrey, C., Maroldt, J., Durst, M., Hoppe-Seyler, F., 2008. Activation of the enhancer of zeste homologue 2 gene by the human papillomavirus E7 oncoprotein. *Cancer research* 68, 9964-9972.
- Holmgren, S.C., Patterson, N.A., Ozbun, M.A., Lambert, P.F., 2005. The minor capsid protein L2 contributes to two steps in the human papillomavirus type 31 life cycle. *Journal of virology* 79, 3938-3948.
- Howie, H.L., Katzenellenbogen, R.A., Galloway, D.A., 2009. Papillomavirus E6 proteins. *Virology* 384, 324-334.
- Huang, P.S., Patrick, D.R., Edwards, G., Goodhart, P.J., Huber, H.E., Miles, L., Garsky, V.M., Oliff, A., Heimbrook, D.C., 1993. Protein domains governing interactions between E2F, the retinoblastoma gene product, and human

- papillomavirus type 16 E7 protein. *Molecular and cellular biology* 13, 953-960.
- Huang, S.M., McCance, D.J., 2002. Down regulation of the interleukin-8 promoter by human papillomavirus type 16 E6 and E7 through effects on CREB binding protein/p300 and P/CAF. *Journal of virology* 76, 8710-8721.
- Hudson, J.B., Bedell, M.A., McCance, D.J., Laiminis, L.A., 1990. Immortalization and altered differentiation of human keratinocytes in vitro by the E6 and E7 open reading frames of human papillomavirus type 18. *Journal of virology* 64, 519-526.
- Huh, K., Zhou, X., Hayakawa, H., Cho, J.Y., Libermann, T.A., Jin, J., Harper, J.W., Munger, K., 2007. Human papillomavirus type 16 E7 oncoprotein associates with the cullin 2 ubiquitin ligase complex, which contributes to degradation of the retinoblastoma tumor suppressor. *Journal of virology* 81, 9737-9747.
- Huh, K.W., DeMasi, J., Ogawa, H., Nakatani, Y., Howley, P.M., Munger, K., 2005. Association of the human papillomavirus type 16 E7 oncoprotein with the 600-kDa retinoblastoma protein-associated factor, p600. *Proceedings of the National Academy of Sciences of the United States of America* 102, 11492-11497.
- Huibregtse, J.M., Scheffner, M., Howley, P.M., 1991. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. *The EMBO journal* 10, 4129-4135.
- Huibregtse, J.M., Scheffner, M., Howley, P.M., 1993a. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. *Molecular and cellular biology* 13, 775-784.
- Huibregtse, J.M., Scheffner, M., Howley, P.M., 1993b. Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. *Molecular and cellular biology* 13, 4918-4927.
- Humbert, P.O., Dow, L.E., Russell, S.M., 2006. The Scribble and Par complexes in polarity and migration: friends or foes? *Trends in cell biology* 16, 622-630.
- Humphries, A.C., Mlodzik, M., 2018. From instruction to output: Wnt/PCP signaling in development and cancer. *Current opinion in cell biology* 51, 110-116.
- Hurlin, P.J., Kaur, P., Smith, P.P., Perez-Reyes, N., Blanton, R.A., McDougall, J.K., 1991. Progression of human papillomavirus type 18-immortalized human keratinocytes to a malignant phenotype. *Proceedings of the*

National Academy of Sciences of the United States of America 88, 570-574.

- Hwang, S.G., Lee, D., Kim, J., Seo, T., Choe, J., 2002. Human papillomavirus type 16 E7 binds to E2F1 and activates E2F1-driven transcription in a retinoblastoma protein-independent manner. *The Journal of biological chemistry* 277, 2923-2930.
- Ide, N., Hata, Y., Nishioka, H., Hirao, K., Yao, I., Deguchi, M., Mizoguchi, A., Nishimori, H., Tokino, T., Nakamura, Y., Takai, Y., 1999. Localization of membrane-associated guanylate kinase (MAGI)-1/BAI-associated protein (BAP) 1 at tight junctions of epithelial cells. *Oncogene* 18, 7810-7815.
- Itoh, Y., 2015. Membrane-type matrix metalloproteinases: Their functions and regulations. *Matrix biology : journal of the International Society for Matrix Biology* 44-46, 207-223.
- Jablonska-Trypuc, A., Matejczyk, M., Rosochacki, S., 2016. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. *Journal of enzyme inhibition and medicinal chemistry* 31, 177-183.
- James, M.A., Lee, J.H., Klingelutz, A.J., 2006. HPV16-E6 associated hTERT promoter acetylation is E6AP dependent, increased in later passage cells and enhanced by loss of p300. *International journal of cancer. Journal international du cancer* 119, 1878-1885.
- Javier, R.T., 2008. Cell polarity proteins: common targets for tumorigenic human viruses. *Oncogene* 27, 7031-7046.
- Jenny, A., Reynolds-Kenneally, J., Das, G., Burnett, M., Mlodzik, M., 2005. Diego and Prickle regulate Frizzled planar cell polarity signalling by competing for Dishevelled binding. *Nature cell biology* 7, 691-697.
- Jeon, J.H., Cho, S.Y., Kim, C.W., Shin, D.M., Jang, G.Y., Lee, H.J., Kang, H.S., Park, S.C., Kim, I.G., 2006. Alteration of Rb binding to HPV 18 E7 modified by transglutaminase 2 with different type of polyamines. *Frontiers in bioscience : a journal and virtual library* 11, 1540-1548.
- Jeon, J.H., Choi, K.H., Cho, S.Y., Kim, C.W., Shin, D.M., Kwon, J.C., Song, K.Y., Park, S.C., Kim, I.G., 2003. Transglutaminase 2 inhibits Rb binding of human papillomavirus E7 by incorporating polyamine. *The EMBO journal* 22, 5273-5282.
- Jha, S., Vande Pol, S., Banerjee, N.S., Dutta, A.B., Chow, L.T., Dutta, A., 2010. Destabilization of TIP60 by human papillomavirus E6 results in attenuation of TIP60-dependent transcriptional regulation and apoptotic pathway. *Molecular cell* 38, 700-711.
- Jian, Y., Schmidt-Grimminger, D.C., Chien, W.M., Wu, X., Broker, T.R., Chow, L.T., 1998. Post-transcriptional induction of p21cip1 protein by human

papillomavirus E7 inhibits unscheduled DNA synthesis reactivated in differentiated keratinocytes. *Oncogene* 17, 2027-2038.

- Johansson, C., Somberg, M., Li, X., Backstrom Winqvist, E., Fay, J., Ryan, F., Pim, D., Banks, L., Schwartz, S., 2012. HPV-16 E2 contributes to induction of HPV-16 late gene expression by inhibiting early polyadenylation. *The EMBO journal* 31, 3212-3227.
- Johansson, N., Vaalamo, M., Grenman, S., Hietanen, S., Klemi, P., Saarialho-Kere, U., Kahari, V.M., 1999. Collagenase-3 (MMP-13) is expressed by tumor cells in invasive vulvar squamous cell carcinomas. *The American journal of pathology* 154, 469-480.
- Johnson, K.M., Kines, R.C., Roberts, J.N., Lowy, D.R., Schiller, J.T., Day, P.M., 2009. Role of heparan sulfate in attachment to and infection of the murine female genital tract by human papillomavirus. *Journal of virology* 83, 2067-2074.
- Jones, D.L., Alani, R.M., Munger, K., 1997a. The human papillomavirus E7 oncoprotein can uncouple cellular differentiation and proliferation in human keratinocytes by abrogating p21Cip1-mediated inhibition of cdk2. *Genes & development* 11, 2101-2111.
- Jones, D.L., Munger, K., 1997. Analysis of the p53-mediated G1 growth arrest pathway in cells expressing the human papillomavirus type 16 E7 oncoprotein. *Journal of virology* 71, 2905-2912.
- Jones, D.L., Thompson, D.A., Munger, K., 1997b. Destabilization of the RB tumor suppressor protein and stabilization of p53 contribute to HPV type 16 E7-induced apoptosis. *Virology* 239, 97-107.
- Jones, D.L., Thompson, D.A., Suh-Burgmann, E., Grace, M., Munger, K., 1999. Expression of the HPV E7 oncoprotein mimics but does not evoke a p53-dependent cellular DNA damage response pathway. *Virology* 258, 406-414.
- Jui, H.Y., Tseng, R.J., Wen, X., Fang, H.I., Huang, L.M., Chen, K.Y., Kung, H.J., Ann, D.K., Shih, H.M., 2000. Protein-tyrosine phosphatase D1, a potential regulator and effector for Tec family kinases. *The Journal of biological chemistry* 275, 41124-41132.
- Kaewprag, J., Umnajvjit, W., Ngamkham, J., Ponglikitmongkol, M., 2013. HPV16 oncoproteins promote cervical cancer invasiveness by upregulating specific matrix metalloproteinases. *PloS one* 8, e71611.
- Kallay, L.M., McNickle, A., Brennwald, P.J., Hubbard, A.L., Braiterman, L.T., 2006. Scribble associates with two polarity proteins, Lgl2 and Vangl2, via distinct molecular domains. *Journal of cellular biochemistry* 99, 647-664.
- Kamio, M., Yoshida, T., Ogata, H., Douchi, T., Nagata, Y., Inoue, M., Hasegawa, M., Yonemitsu, Y., Yoshimura, A., 2004. SOCS1 [corrected] inhibits HPV-

E7-mediated transformation by inducing degradation of E7 protein. *Oncogene* 23, 3107-3115.

- Kan, Q., Jinno, S., Yamamoto, H., Kobayashi, K., Okayama, H., 2008. ATP-dependent activation of p21WAF1/CIP1-associated Cdk2 by Cdc6. *Proceedings of the National Academy of Sciences of the United States of America* 105, 4757-4762.
- Kanda, T., Furuno, A., Yoshiike, K., 1988. Human papillomavirus type 16 open reading frame E7 encodes a transforming gene for rat 3Y1 cells. *Journal of virology* 62, 610-613.
- Katich, S.C., Zeffass-Thome, K., Hoffmann, I., 2001. Regulation of the Cdc25A gene by the human papillomavirus Type 16 E7 oncogene. *Oncogene* 20, 543-550.
- Kelly, L.K., Wu, J., Yanfeng, W.A., Mlodzik, M., 2016. Frizzled-Induced Van Gogh Phosphorylation by CK1epsilon Promotes Asymmetric Localization of Core PCP Factors in Drosophila. *Cell reports* 16, 344-356.
- Kharfallah, F., Guyot, M.C., El Hassan, A.R., Allache, R., Merello, E., De Marco, P., Di Cristo, G., Capra, V., Kibar, Z., 2017. Scribble1 plays an important role in the pathogenesis of neural tube defects through its mediating effect of Par-3 and Vangl1/2 localization. *Human molecular genetics* 26, 2307-2320.
- Kibar, Z., Torban, E., McDearmid, J.R., Reynolds, A., Berghout, J., Mathieu, M., Kirillova, I., De Marco, P., Merello, E., Hayes, J.M., Wallingford, J.B., Drapeau, P., Capra, V., Gros, P., 2007. Mutations in VANGL1 associated with neural-tube defects. *The New England journal of medicine* 356, 1432-1437.
- Kibar, Z., Vogan, K.J., Groulx, N., Justice, M.J., Underhill, D.A., Gros, P., 2001. Ltap, a mammalian homolog of Drosophila Strabismus/Van Gogh, is altered in the mouse neural tube mutant Loop-tail. *Nature genetics* 28, 251-255.
- Kiyono, T., Hiraiwa, A., Fujita, M., Hayashi, Y., Akiyama, T., Ishibashi, M., 1997. Binding of high-risk human papillomavirus E6 oncoproteins to the human homologue of the Drosophila discs large tumor suppressor protein. *Proceedings of the National Academy of Sciences of the United States of America* 94, 11612-11616.
- Klein, T., Bischoff, R., 2011. Physiology and pathophysiology of matrix metalloproteases. *Amino Acids* 41, 271-290.
- Klingelhutz, A.J., Barber, S.A., Smith, P.P., Dyer, K., McDougall, J.K., 1994. Restoration of telomeres in human papillomavirus-immortalized human anogenital epithelial cells. *Molecular and cellular biology* 14, 961-969.
- Klingelhutz, A.J., Foster, S.A., McDougall, J.K., 1996. Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* 380, 79-82.

- Knapp, A.A., McManus, P.M., Bockstall, K., Moroianu, J., 2009. Identification of the nuclear localization and export signals of high risk HPV16 E7 oncoprotein. *Virology* 383, 60-68.
- Korpi, J.T., Kervinen, V., Maklin, H., Vaananen, A., Lahtinen, M., Laara, E., Ristimäki, A., Thomas, G., Ylipalosaari, M., Astrom, P., Lopez-Otin, C., Sorsa, T., Kantola, S., Pirila, E., Salo, T., 2008. Collagenase-2 (matrix metalloproteinase-8) plays a protective role in tongue cancer. *British journal of cancer* 98, 766-775.
- Korzeniewski, N., Treat, B., Duensing, S., 2011. The HPV-16 E7 oncoprotein induces centriole multiplication through deregulation of Polo-like kinase 4 expression. *Molecular cancer* 10, 61.
- Kranjec, C., Holleywood, C., Libert, D., Griffin, H., Mahmood, R., Isaacson, E., Doorbar, J., 2017. Modulation of basal cell fate during productive and transforming HPV-16 infection is mediated by progressive E6-driven depletion of Notch. *The Journal of pathology* 242, 448-462.
- Kuballa, P., Matentzoglou, K., Scheffner, M., 2007. The role of the ubiquitin ligase E6-AP in human papillomavirus E6-mediated degradation of PDZ domain-containing proteins. *The Journal of biological chemistry* 282, 65-71.
- Kuhne, C., Gardiol, D., Guarnaccia, C., Amenitsch, H., Banks, L., 2000. Differential regulation of human papillomavirus E6 by protein kinase A: conditional degradation of human discs large protein by oncogenic E6. *Oncogene* 19, 5884-5891.
- Kukic, P., Lo Piccolo, G.M., Nogueira, M.O., Svergun, D.I., Vendruscolo, M., Felli, I.C., Pierattelli, R., 2019. The free energy landscape of the oncogene protein E7 of human papillomavirus type 16 reveals a complex interplay between ordered and disordered regions. *Scientific reports* 9, 5822.
- Kumar, A., Zhao, Y., Meng, G., Zeng, M., Srinivasan, S., Delmolino, L.M., Gao, Q., Dimri, G., Weber, G.F., Wazer, D.E., Band, H., Band, V., 2002. Human papillomavirus oncoprotein E6 inactivates the transcriptional coactivator human ADA3. *Molecular and cellular biology* 22, 5801-5812.
- Kurayoshi, M., Oue, N., Yamamoto, H., Kishida, M., Inoue, A., Asahara, T., Yasui, W., Kikuchi, A., 2006. Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. *Cancer research* 66, 10439-10448.
- Kuzmichev, A., Margueron, R., Vaquero, A., Preissner, T.S., Scher, M., Kirmizis, A., Ouyang, X., Brockdorff, N., Abate-Shen, C., Farnham, P., Reinberg, D., 2005. Composition and histone substrates of polycomb repressive group complexes change during cellular differentiation. *Proceedings of the National Academy of Sciences of the United States of America* 102, 1859-1864.

- Kwart, D., Paquet, D., Teo, S., Tessier-Lavigne, M., 2017. Precise and efficient scarless genome editing in stem cells using CORRECT. *Nature protocols* 12, 329-354.
- Landesman-Bollag, E., Romieu-Mourez, R., Song, D.H., Sonenshein, G.E., Cardiff, R.D., Seldin, D.C., 2001. Protein kinase CK2 in mammary gland tumorigenesis. *Oncogene* 20, 3247-3257.
- Lechner, M.S., Laimins, L.A., 1994. Inhibition of p53 DNA binding by human papillomavirus E6 proteins. *Journal of virology* 68, 4262-4273.
- Lee, J.H., Park, S.R., Chay, K.O., Seo, Y.W., Kook, H., Ahn, K.Y., Kim, Y.J., Kim, K.K., 2004. KAI1 COOH-terminal interacting tetraspanin (KITENIN), a member of the tetraspanin family, interacts with KAI1, a tumor metastasis suppressor, and enhances metastasis of cancer. *Cancer research* 64, 4235-4243.
- Lee, J.K., Bae, J.A., Sun, E.G., Kim, H.D., Yoon, T.M., Kim, K., Lee, J.H., Lim, S.C., Kim, K.K., 2009. KITENIN increases invasion and migration of mouse squamous cancer cells and promotes pulmonary metastasis in a mouse squamous tumor model. *FEBS letters* 583, 711-717.
- Lee, J.O., Russo, A.A., Pavletich, N.P., 1998. Structure of the retinoblastoma tumour-suppressor pocket domain bound to a peptide from HPV E7. *Nature* 391, 859-865.
- Lee, S.S., Weiss, R.S., Javier, R.T., 1997. Binding of human virus oncoproteins to hDlg/SAP97, a mammalian homolog of the Drosophila discs large tumor suppressor protein. *Proceedings of the National Academy of Sciences of the United States of America* 94, 6670-6675.
- Li, X., Tran, K.M., Aziz, K.E., Sorokin, A.V., Chen, J., Wang, W., 2016. Defining the Protein-Protein Interaction Network of the Human Protein Tyrosine Phosphatase Family. *Molecular & cellular proteomics : MCP* 15, 3030-3044.
- Liang, Y.J., Chang, H.S., Wang, C.Y., Yu, W.C., 2008. DYRK1A stabilizes HPV16E7 oncoprotein through phosphorylation of the threonine 5 and threonine 7 residues. *The international journal of biochemistry & cell biology* 40, 2431-2441.
- Lichtig, H., Gilboa, D.A., Jackman, A., Gonen, P., Levav-Cohen, Y., Haupt, Y., Sherman, L., 2010. HPV16 E6 augments Wnt signaling in an E6AP-dependent manner. *Virology* 396, 47-58.
- Lin, C.H., Chang, H.S., Yu, W.C., 2008. USP11 stabilizes HPV-16E7 and further modulates the E7 biological activity. *The Journal of biological chemistry* 283, 15681-15688.
- Lin, H.Y., Chen, Y.S., Wang, K., Chien, H.W., Hsieh, Y.H., Yang, S.F., 2017. Fisetin inhibits epidermal growth factor-induced migration of ARPE-19

- cells by suppression of AKT activation and Sp1-dependent MMP-9 expression. *Molecular vision* 23, 900-910.
- Lin, S., Staahl, B.T., Alla, R.K., Doudna, J.A., 2014. Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *eLife* 3, e04766.
- Litchfield, D.W., 2003. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *The Biochemical journal* 369, 1-15.
- Liu, X., Clements, A., Zhao, K., Marmorstein, R., 2006. Structure of the human Papillomavirus E7 oncoprotein and its mechanism for inactivation of the retinoblastoma tumor suppressor. *The Journal of biological chemistry* 281, 578-586.
- Liu, X., Dakic, A., Zhang, Y., Dai, Y., Chen, R., Schlegel, R., 2009. HPV E6 protein interacts physically and functionally with the cellular telomerase complex. *Proceedings of the National Academy of Sciences of the United States of America* 106, 18780-18785.
- Liu, X., Yang, N., Figel, S.A., Wilson, K.E., Morrison, C.D., Gelman, I.H., Zhang, J., 2013. PTPN14 interacts with and negatively regulates the oncogenic function of YAP. *Oncogene* 32, 1266-1273.
- Loffek, S., Schilling, O., Franzke, C.W., 2011. Series "matrix metalloproteinases in lung health and disease": Biological role of matrix metalloproteinases: a critical balance. *Eur Respir J* 38, 191-208.
- Longworth, M.S., Wilson, R., Laimins, L.A., 2005. HPV31 E7 facilitates replication by activating E2F2 transcription through its interaction with HDACs. *The EMBO journal* 24, 1821-1830.
- Lorand, L., Graham, R.M., 2003. Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nature Reviews Molecular Cell Biology* 4, 140-156.
- Lu, Z., Hu, X., Li, Y., Zheng, L., Zhou, Y., Jiang, H., Ning, T., Basang, Z., Zhang, C., Ke, Y., 2004. Human papillomavirus 16 E6 oncoprotein interferences with insulin signaling pathway by binding to tuberlin. *The Journal of biological chemistry* 279, 35664-35670.
- Luga, V., Zhang, L., Vitoria-Petit, A.M., Ogunjimi, A.A., Inanlou, M.R., Chiu, E., Buchanan, M., Hosein, A.N., Basik, M., Wrana, J.L., 2012. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* 151, 1542-1556.
- Lyons, T.E., Salih, M., Tuana, B.S., 2006. Activating E2Fs mediate transcriptional regulation of human E2F6 repressor. *American journal of physiology. Cell physiology* 290, C189-199.
- Mantovani, F., Banks, L., 1999. Inhibition of E6 induced degradation of p53 is not sufficient for stabilization of p53 protein in cervical tumour derived cell lines. *Oncogene* 18, 3309-3315.

- Manzo-Merino, J., Thomas, M., Fuentes-Gonzalez, A.M., Lizano, M., Banks, L., 2013. HPV E6 oncoprotein as a potential therapeutic target in HPV related cancers. *Expert opinion on therapeutic targets* 17, 1357-1368.
- Marin, O., Meggio, F., Marchiori, F., Borin, G., Pinna, L.A., 1986. Site specificity of casein kinase-2 (TS) from rat liver cytosol. A study with model peptide substrates. *European journal of biochemistry / FEBS* 160, 239-244.
- Marin, O., Meggio, F., Pinna, L.A., 1994. Design and synthesis of two new peptide substrates for the specific and sensitive monitoring of casein kinases-1 and -2. *Biochemical and biophysical research communications* 198, 898-905.
- Martini, M., De Santis, M.C., Braccini, L., Gulluni, F., Hirsch, E., 2014. PI3K/AKT signaling pathway and cancer: an updated review. *Annals of medicine* 46, 372-383.
- Maruyama, T., Dougan, S.K., Truttmann, M.C., Bilate, A.M., Ingram, J.R., Ploegh, H.L., 2015. Increasing the efficiency of precise genome editing with CRISPR-Cas9 by inhibition of nonhomologous end joining. *Nature biotechnology* 33, 538-542.
- Massimi, P., Banks, L., 1997. Repression of p53 transcriptional activity by the HPV E7 proteins. *Virology* 227, 255-259.
- Massimi, P., Banks, L., 2000. Differential phosphorylation of the HPV-16 E7 oncoprotein during the cell cycle. *Virology* 276, 388-394.
- Massimi, P., Pim, D., Storey, A., Banks, L., 1996. HPV-16 E7 and adenovirus E1a complex formation with TATA box binding protein is enhanced by casein kinase II phosphorylation. *Oncogene* 12, 2325-2330.
- Massimi, P., Zori, P., Roberts, S., Banks, L., 2012. Differential regulation of cell-cell contact, invasion and anoikis by hScrib and hDlg in keratinocytes. *PloS one* 7, e40279.
- Matis, M., Russler-Germain, D.A., Hu, Q., Tomlin, C.J., Axelrod, J.D., 2014. Microtubules provide directional information for core PCP function. *eLife* 3, e02893.
- Matlashewski, G., Schneider, J., Banks, L., Jones, N., Murray, A., Crawford, L., 1987. Human papillomavirus type 16 DNA cooperates with activated ras in transforming primary cells. *The EMBO journal* 6, 1741-1746.
- McBride, A.A., 2008. Replication and partitioning of papillomavirus genomes. *Advances in virus research* 72, 155-205.
- McCaffrey, L.M., Macara, I.G., 2011. Epithelial organization, cell polarity and tumorigenesis. *Trends in cell biology* 21, 727-735.
- McCance, D.J., Kopan, R., Fuchs, E., Laimins, L.A., 1988. Human papillomavirus type 16 alters human epithelial cell differentiation in vitro. *Proceedings of*

the National Academy of Sciences of the United States of America 85, 7169-7173.

- McIntyre, M.C., Frattini, M.G., Grossman, S.R., Laimins, L.A., 1993. Human papillomavirus type 18 E7 protein requires intact Cys-X-X-Cys motifs for zinc binding, dimerization, and transformation but not for Rb binding. *Journal of virology* 67, 3142-3150.
- McLaughlin-Drubin, M.E., Crum, C.P., Munger, K., 2011. Human papillomavirus E7 oncoprotein induces KDM6A and KDM6B histone demethylase expression and causes epigenetic reprogramming. *Proceedings of the National Academy of Sciences of the United States of America* 108, 2130-2135.
- McLaughlin-Drubin, M.E., Huh, K.W., Munger, K., 2008. Human papillomavirus type 16 E7 oncoprotein associates with E2F6. *Journal of virology* 82, 8695-8705.
- McLaughlin-Drubin, M.E., Munger, K., 2013. Biochemical and functional interactions of human papillomavirus proteins with polycomb group proteins. *Viruses* 5, 1231-1249.
- McLaughlin-Drubin, M.E., Park, D., Munger, K., 2013. Tumor suppressor p16INK4A is necessary for survival of cervical carcinoma cell lines. *Proceedings of the National Academy of Sciences of the United States of America* 110, 16175-16180.
- Melar-New, M., Laimins, L.A., 2010. Human papillomaviruses modulate expression of microRNA 203 upon epithelial differentiation to control levels of p63 proteins. *Journal of virology* 84, 5212-5221.
- Menges, C.W., Baglia, L.A., Lapoint, R., McCance, D.J., 2006. Human papillomavirus type 16 E7 up-regulates AKT activity through the retinoblastoma protein. *Cancer research* 66, 5555-5559.
- Merte, J., Jensen, D., Wright, K., Sarsfield, S., Wang, Y., Schekman, R., Ginty, D.D., 2010. Sec24b selectively sorts Vangl2 to regulate planar cell polarity during neural tube closure. *Nature cell biology* 12, 41-46; sup pp 41-48.
- Middleton, K., Peh, W., Southern, S., Griffin, H., Sotlar, K., Nakahara, T., El-Sherif, A., Morris, L., Seth, R., Hibma, M., Jenkins, D., Lambert, P., Coleman, N., Doorbar, J., 2003. Organization of human papillomavirus productive cycle during neoplastic progression provides a basis for selection of diagnostic markers. *Journal of virology* 77, 10186-10201.
- Mietz, J.A., Unger, T., Huibregtse, J.M., Howley, P.M., 1992. The transcriptional transactivation function of wild-type p53 is inhibited by SV40 large T-antigen and by HPV-16 E6 oncoprotein. *The EMBO journal* 11, 5013-5020.

- Milligan, S.G., Veerapraditsin, T., Ahamet, B., Mole, S., Graham, S.V., 2007. Analysis of novel human papillomavirus type 16 late mRNAs in differentiated W12 cervical epithelial cells. *Virology* 360, 172-181.
- Moller, N.P., Moller, K.B., Lammers, R., Kharitononkov, A., Sures, I., Ullrich, A., 1994. Src kinase associates with a member of a distinct subfamily of protein-tyrosine phosphatases containing an ezrin-like domain. *Proceedings of the National Academy of Sciences of the United States of America* 91, 7477-7481.
- Montagnoli, A., Fiore, F., Eytan, E., Carrano, A.C., Draetta, G.F., Hershko, A., Pagano, M., 1999. Ubiquitination of p27 is regulated by Cdk-dependent phosphorylation and trimeric complex formation. *Genes & development* 13, 1181-1189.
- Moody, C.A., Laimins, L.A., 2009. Human papillomaviruses activate the ATM DNA damage pathway for viral genome amplification upon differentiation. *PLoS pathogens* 5, e1000605.
- Moody, C.A., Laimins, L.A., 2010. Human papillomavirus oncoproteins: pathways to transformation. *Nature reviews. Cancer* 10, 550-560.
- Moore, P.S., Chang, Y., 2010. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nature reviews. Cancer* 10, 878-889.
- Muller, C., Alunni-Fabbroni, M., Kowenz-Leutz, E., Mo, X., Tommasino, M., Leutz, A., 1999. Separation of C/EBPalpha-mediated proliferation arrest and differentiation pathways. *Proceedings of the National Academy of Sciences of the United States of America* 96, 7276-7281.
- Munger, K., Gwin, T.K., McLaughlin-Drubin, M.E., 2013. p16 in HPV-associated cancers. *Oncotarget* 4, 1864-1865.
- Munger, K., Phelps, W.C., Bubb, V., Howley, P.M., Schlegel, R., 1989a. The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. *Journal of virology* 63, 4417-4421.
- Munger, K., Werness, B.A., Dyson, N., Phelps, W.C., Harlow, E., Howley, P.M., 1989b. Complex formation of human papillomavirus E7 proteins with the retinoblastoma tumor suppressor gene product. *The EMBO journal* 8, 4099-4105.
- Murao, S., Collart, F.R., Huberman, E., 1989. A protein containing the cystic fibrosis antigen is an inhibitor of protein kinases. *The Journal of biological chemistry* 264, 8356-8360.
- Murdoch, J.N., Doudney, K., Paternotte, C., Copp, A.J., Stanier, P., 2001. Severe neural tube defects in the loop-tail mouse result from mutation of Lpp1, a novel gene involved in floor plate specification. *Human molecular genetics* 10, 2593-2601.

- Murray-Zmijewski, F., Slee, E.A., Lu, X., 2008. A complex barcode underlies the heterogeneous response of p53 to stress. *Nature reviews. Molecular cell biology* 9, 702-712.
- Mutirangura, A., Sriuranpong, V., Termrunggraunglert, W., Tresukosol, D., Lertsaguansinchai, P., Voravud, N., Niruthisard, S., 1998. Telomerase activity and human papillomavirus in malignant, premalignant and benign cervical lesions. *British journal of cancer* 78, 933-939.
- Nakagawa, S., Huibregtse, J.M., 2000. Human scribble (Vartul) is targeted for ubiquitin-mediated degradation by the high-risk papillomavirus E6 proteins and the E6AP ubiquitin-protein ligase. *Molecular and cellular biology* 20, 8244-8253.
- Nees, M., Geoghegan, J.M., Munson, P., Prabhu, V., Liu, Y., Androphy, E., Woodworth, C.D., 2000. Human papillomavirus type 16 E6 and E7 proteins inhibit differentiation-dependent expression of transforming growth factor-beta2 in cervical keratinocytes. *Cancer research* 60, 4289-4298.
- Nguyen, C.L., Eichwald, C., Nibert, M.L., Munger, K., 2007. Human papillomavirus type 16 E7 oncoprotein associates with the centrosomal component gamma-tubulin. *Journal of virology* 81, 13533-13543.
- Nguyen, C.L., McLaughlin-Drubin, M.E., Munger, K., 2008. Delocalization of the microtubule motor Dynein from mitotic spindles by the human papillomavirus E7 oncoprotein is not sufficient for induction of multipolar mitoses. *Cancer research* 68, 8715-8722.
- Nguyen, C.L., Munger, K., 2008. Direct association of the HPV16 E7 oncoprotein with cyclin A/CDK2 and cyclin E/CDK2 complexes. *Virology* 380, 21-25.
- Nguyen, C.L., Munger, K., 2009. Human papillomavirus E7 protein deregulates mitosis via an association with nuclear mitotic apparatus protein 1. *Journal of virology* 83, 1700-1707.
- Nguyen, D.X., Westbrook, T.F., McCance, D.J., 2002. Human papillomavirus type 16 E7 maintains elevated levels of the cdc25A tyrosine phosphatase during deregulation of cell cycle arrest. *Journal of virology* 76, 619-632.
- Ni, F., Yu, W.M., Wang, X., Fay, M.E., Young, K.M., Qiu, Y., Lam, W.A., Sulchek, T.A., Cheng, T., Scadden, D.T., Qu, C.K., 2019. Ptpn21 Controls Hematopoietic Stem Cell Homeostasis and Biomechanics. *Cell stem cell* 24, 608-620 e606.
- Nogueira, M.O., Hosek, T., Calcada, E.O., Castiglia, F., Massimi, P., Banks, L., Felli, I.C., Pierattelli, R., 2017. Monitoring HPV-16 E7 phosphorylation events. *Virology* 503, 70-75.
- Nomine, Y., Masson, M., Charbonnier, S., Zanier, K., Ristriani, T., Deryckere, F., Sibler, A.P., Desplancq, D., Atkinson, R.A., Weiss, E., Orfanoudakis, G., Kieffer, B., Trave, G., 2006. Structural and functional analysis of E6

- oncoprotein: insights in the molecular pathways of human papillomavirus-mediated pathogenesis. *Molecular cell* 21, 665-678.
- Noya, F., Chien, W.M., Broker, T.R., Chow, L.T., 2001. p21cip1 Degradation in differentiated keratinocytes is abrogated by costabilization with cyclin E induced by human papillomavirus E7. *Journal of virology* 75, 6121-6134.
- Oh, K.J., Kalinina, A., Wang, J., Nakayama, K., Nakayama, K.I., Bagchi, S., 2004. The papillomavirus E7 oncoprotein is ubiquitinated by UbchH7 and Cullin 1- and Skp2-containing E3 ligase. *Journal of virology* 78, 5338-5346.
- Oh, S.T., Kyo, S., Laimins, L.A., 2001. Telomerase activation by human papillomavirus type 16 E6 protein: induction of human telomerase reverse transcriptase expression through Myc and GC-rich Sp1 binding sites. *Journal of virology* 75, 5559-5566.
- Ohlenschlager, O., Seiboth, T., Zengerling, H., Briese, L., Marchanka, A., Ramachandran, R., Baum, M., Korbass, M., Meyer-Klaucke, W., Durst, M., Grollach, M., 2006. Solution structure of the partially folded high-risk human papilloma virus 45 oncoprotein E7. *Oncogene* 25, 5953-5959.
- Ohta, T., Xiong, Y., 2001. Phosphorylation- and Skp1-independent in vitro ubiquitination of E2F1 by multiple ROC-cullin ligases. *Cancer research* 61, 1347-1353.
- Ortega, C.E., Seidner, Y., Dominguez, I., 2014. Mining CK2 in cancer. *PloS one* 9, e115609.
- Ozbun, M.A., Meyers, C., 1998. Human papillomavirus type 31b E1 and E2 transcript expression correlates with vegetative viral genome amplification. *Virology* 248, 218-230.
- Pagano, M.A., Meggio, F., Ruzzene, M., Andrzejewska, M., Kazimierczuk, Z., Pinna, L.A., 2004. 2-Dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole: a novel powerful and selective inhibitor of protein kinase CK2. *Biochemical and biophysical research communications* 321, 1040-1044.
- Paquet, D., Kwart, D., Chen, A., Sproul, A., Jacob, S., Teo, S., Olsen, K.M., Gregg, A., Noggle, S., Tessier-Lavigne, M., 2016. Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. *Nature* 533, 125-129.
- Park, J.S., Kim, E.J., Kwon, H.J., Hwang, E.S., Namkoong, S.E., Um, S.J., 2000. Inactivation of interferon regulatory factor-1 tumor suppressor protein by HPV E7 oncoprotein. Implication for the E7-mediated immune evasion mechanism in cervical carcinogenesis. *The Journal of biological chemistry* 275, 6764-6769.

- Patel, D., Huang, S.M., Baglia, L.A., McCance, D.J., 1999. The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300. *The EMBO journal* 18, 5061-5072.
- Pei, X.F., Meck, J.M., Greenhalgh, D., Schlegel, R., 1993. Cotransfection of HPV-18 and v-fos DNA induces tumorigenicity of primary human keratinocytes. *Virology* 196, 855-860.
- Perea, S.E., Baladron, I., Valenzuela, C., Perera, Y., 2018. CIGB-300: A peptide-based drug that impairs the Protein Kinase CK2-mediated phosphorylation. *Seminars in oncology* 45, 58-67.
- Perea, S.E., Reyes, O., Baladron, I., Perera, Y., Farina, H., Gil, J., Rodriguez, A., Bacardi, D., Marcelo, J.L., Cosme, K., Cruz, M., Valenzuela, C., Lopez-Saura, P.A., Puchades, Y., Serrano, J.M., Mendoza, O., Castellanos, L., Sanchez, A., Betancourt, L., Besada, V., Silva, R., Lopez, E., Falcon, V., Hernandez, I., Solares, M., Santana, A., Diaz, A., Ramos, T., Lopez, C., Ariosa, J., Gonzalez, L.J., Garay, H., Gomez, D., Gomez, R., Alonso, D.F., Sigman, H., Herrera, L., Acevedo, B., 2008. CIGB-300, a novel proapoptotic peptide that impairs the CK2 phosphorylation and exhibits anticancer properties both in vitro and in vivo. *Molecular and cellular biochemistry* 316, 163-167.
- Perea, S.E., Reyes, O., Puchades, Y., Mendoza, O., Vispo, N.S., Torrens, I., Santos, A., Silva, R., Acevedo, B., Lopez, E., Falcon, V., Alonso, D.F., 2004. Antitumor effect of a novel proapoptotic peptide that impairs the phosphorylation by the protein kinase 2 (casein kinase 2). *Cancer research* 64, 7127-7129.
- Perera, Y., Farina, H.G., Hernandez, I., Mendoza, O., Serrano, J.M., Reyes, O., Gomez, D.E., Gomez, R.E., Acevedo, B.E., Alonso, D.F., Perea, S.E., 2008. Systemic administration of a peptide that impairs the protein kinase (CK2) phosphorylation reduces solid tumor growth in mice. *International journal of cancer. Journal international du cancer* 122, 57-62.
- Phelps, W.C., Munger, K., Yee, C.L., Barnes, J.A., Howley, P.M., 1992. Structure-function analysis of the human papillomavirus type 16 E7 oncoprotein. *Journal of virology* 66, 2418-2427.
- Phelps, W.C., Yee, C.L., Munger, K., Howley, P.M., 1988. The human papillomavirus type 16 E7 gene encodes transactivation and transformation functions similar to those of adenovirus E1A. *Cell* 53, 539-547.
- Phillips, A.C., Vousden, K.H., 1997. Analysis of the interaction between human papillomavirus type 16 E7 and the TATA-binding protein, TBP. *The Journal of general virology* 78 (Pt 4), 905-909.
- Piazza, F.A., Ruzzene, M., Gurrieri, C., Montini, B., Bonanni, L., Chioetto, G., Di Maira, G., Barbon, F., Cabrelle, A., Zambello, R., Adami, F., Trentin, L.,

- Pinna, L.A., Semenzato, G., 2006. Multiple myeloma cell survival relies on high activity of protein kinase CK2. *Blood* 108, 1698-1707.
- Pickard, A., Cichon, A.C., Barry, A., Kieran, D., Patel, D., Hamilton, P., Salto-Tellez, M., James, J., McCance, D.J., 2012. Inactivation of Rb in stromal fibroblasts promotes epithelial cell invasion. *The EMBO journal* 31, 3092-3103.
- Pierre, F., Chua, P.C., O'Brien, S.E., Siddiqui-Jain, A., Bourbon, P., Haddach, M., Michaux, J., Nagasawa, J., Schwaebe, M.K., Stefan, E., Vialettes, A., Whitten, J.P., Chen, T.K., Darjania, L., Stansfield, R., Anderes, K., Bliesath, J., Drygin, D., Ho, C., Omori, M., Proffitt, C., Streiner, N., Trent, K., Rice, W.G., Ryckman, D.M., 2011. Discovery and SAR of 5-(3-chlorophenylamino)benzo[c][2,6]naphthyridine-8-carboxylic acid (CX-4945), the first clinical stage inhibitor of protein kinase CK2 for the treatment of cancer. *Journal of medicinal chemistry* 54, 635-654.
- Piirsoo, A., Piirsoo, M., Kala, M., Sankovski, E., Lototskaja, E., Levin, V., Salvi, M., Ustav, M., 2019. Activity of CK2 α protein kinase is required for efficient replication of some HPV types. *PLoS pathogens* 15, e1007788.
- Pim, D., Banks, L., 1999. HPV-18 E6* protein modulates the E6-directed degradation of p53 by binding to full-length HPV-18 E6. *Oncogene* 18, 7403-7408.
- Pim, D., Bergant, M., Boon, S.S., Ganti, K., Kranjec, C., Massimi, P., Subbaiah, V.K., Thomas, M., Tomaic, V., Banks, L., 2012. Human papillomaviruses and the specificity of PDZ domain targeting. *The FEBS journal* 279, 3530-3537.
- Pim, D., Massimi, P., Banks, L., 1997. Alternatively spliced HPV-18 E6* protein inhibits E6 mediated degradation of p53 and suppresses transformed cell growth. *Oncogene* 15, 257-264.
- Pim, D., Massimi, P., Dilworth, S.M., Banks, L., 2005. Activation of the protein kinase B pathway by the HPV-16 E7 oncoprotein occurs through a mechanism involving interaction with PP2A. *Oncogene* 24, 7830-7838.
- Pinna, L.A., 2002. Protein kinase CK2: a challenge to canons. *Journal of cell science* 115, 3873-3878.
- Plummer, M., de Martel, C., Vignat, J., Ferlay, J., Bray, F., Franceschi, S., 2016. Global burden of cancers attributable to infections in 2012: a synthetic analysis. *The Lancet. Global health* 4, e609-616.
- Poirson, J., Biquand, E., Straub, M.L., Cassonnet, P., Nomine, Y., Jones, L., van der Werf, S., Trave, G., Zanier, K., Jacob, Y., Demeret, C., Masson, M., 2017. Mapping the interactome of HPV E6 and E7 oncoproteins with the Ubiquitin-Proteasome System. *The FEBS journal*.
- Psyrris, A., DeFilippis, R.A., Edwards, A.P., Yates, K.E., Manuelidis, L., DiMaio, D., 2004. Role of the retinoblastoma pathway in senescence triggered by

- repression of the human papillomavirus E7 protein in cervical carcinoma cells. *Cancer research* 64, 3079-3086.
- Pukrop, T., Klemm, F., Hagemann, T., Gradl, D., Schulz, M., Siemes, S., Trumper, L., Binder, C., 2006. Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proceedings of the National Academy of Sciences of the United States of America* 103, 5454-5459.
- Pyeon, D., Pearce, S.M., Lank, S.M., Ahlquist, P., Lambert, P.F., 2009. Establishment of human papillomavirus infection requires cell cycle progression. *PLoS pathogens* 5, e1000318.
- Rabalski, A.J., Gyenis, L., Litchfield, D.W., 2016. Molecular Pathways: Emergence of Protein Kinase CK2 (CSNK2) as a Potential Target to Inhibit Survival and DNA Damage Response and Repair Pathways in Cancer Cells. *Clinical cancer research : an official journal of the American Association for Cancer Research* 22, 2840-2847.
- Rampias, T., Boutati, E., Pectasides, E., Sasaki, C., Kountourakis, P., Weinberger, P., Psyrri, A., 2010. Activation of Wnt signaling pathway by human papillomavirus E6 and E7 oncogenes in HPV16-positive oropharyngeal squamous carcinoma cells. *Molecular cancer research : MCR* 8, 433-443.
- Ran, F.A., Hsu, P.D., Wright, J., Agarwala, V., Scott, D.A., Zhang, F., 2013. Genome engineering using the CRISPR-Cas9 system. *Nature protocols* 8, 2281-2308.
- Reinstein, E., Scheffner, M., Oren, M., Ciechanover, A., Schwartz, A., 2000. Degradation of the E7 human papillomavirus oncoprotein by the ubiquitin-proteasome system: targeting via ubiquitination of the N-terminal residue. *Oncogene* 19, 5944-5950.
- Rey, O., Lee, S., Baluda, M.A., Swee, J., Ackerson, B., Chiu, R., Park, N.H., 2000. The E7 oncoprotein of human papillomavirus type 16 interacts with F-actin in vitro and in vivo. *Virology* 268, 372-381.
- Roden, R.B.S., Stern, P.L., 2018. Opportunities and challenges for human papillomavirus vaccination in cancer. *Nature reviews. Cancer* 18, 240-254.
- Rohani, M.G., Parks, W.C., 2015. Matrix remodeling by MMPs during wound repair. *Matrix biology : journal of the International Society for Matrix Biology* 44-46, 113-121.
- Roman, A., 2006. The human papillomavirus E7 protein shines a spotlight on the pRB family member, p130. *Cell cycle* 5, 567-568.
- Rozenblatt-Rosen, O., Deo, R.C., Padi, M., Adelmant, G., Calderwood, M.A., Rolland, T., Grace, M., Dricot, A., Askenazi, M., Tavares, M., Pevzner, S.J., Abderazzaq, F., Byrdsong, D., Carvunis, A.R., Chen, A.A., Cheng, J., Correll, M., Duarte, M., Fan, C., Feltkamp, M.C., Ficarro, S.B., Franchi,

- R., Garg, B.K., Gulbahce, N., Hao, T., Holthaus, A.M., James, R., Korkhin, A., Litovchick, L., Mar, J.C., Pak, T.R., Rabello, S., Rubio, R., Shen, Y., Singh, S., Spangle, J.M., Tasan, M., Wanamaker, S., Webber, J.T., Roecklein-Canfield, J., Johannsen, E., Barabasi, A.L., Beroukhim, R., Kieff, E., Cusick, M.E., Hill, D.E., Munger, K., Marto, J.A., Quackenbush, J., Roth, F.P., DeCaprio, J.A., Vidal, M., 2012. Interpreting cancer genomes using systematic host network perturbations by tumour virus proteins. *Nature* 487, 491-495.
- Ruesch, M.N., Laimins, L.A., 1997. Initiation of DNA synthesis by human papillomavirus E7 oncoproteins is resistant to p21-mediated inhibition of cyclin E-cdk2 activity. *Journal of virology* 71, 5570-5578.
- Ryu, H.S., Park, Y.L., Park, S.J., Lee, J.H., Cho, S.B., Lee, W.S., Chung, I.J., Kim, K.K., Lee, K.H., Kweon, S.S., Joo, Y.E., 2010. KITENIN is associated with tumor progression in human gastric cancer. *Anticancer research* 30, 3479-3486.
- Sakakibara, N., Mitra, R., McBride, A.A., 2011. The papillomavirus E1 helicase activates a cellular DNA damage response in viral replication foci. *Journal of virology* 85, 8981-8995.
- Saleh-Gohari, N., Helleday, T., 2004. Conservative homologous recombination preferentially repairs DNA double-strand breaks in the S phase of the cell cycle in human cells. *Nucleic acids research* 32, 3683-3688.
- Sapp, M., Volpers, C., Muller, M., Streeck, R.E., 1995. Organization of the major and minor capsid proteins in human papillomavirus type 33 virus-like particles. *The Journal of general virology* 76 (Pt 9), 2407-2412.
- Sarno, S., Vaglio, P., Meggio, F., Issinger, O.G., Pinna, L.A., 1996. Protein kinase CK2 mutants defective in substrate recognition. Purification and kinetic analysis. *The Journal of biological chemistry* 271, 10595-10601.
- Sato, H., Watanabe, S., Furuno, A., Yoshiike, K., 1989. Human papillomavirus type 16 E7 protein expressed in *Escherichia coli* and monkey COS-1 cells: immunofluorescence detection of the nuclear E7 protein. *Virology* 170, 311-315.
- Scheffner, M., Werness, B.A., Huibregtse, J.M., Levine, A.J., Howley, P.M., 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell* 63, 1129-1136.
- Schelhaas, M., Shah, B., Holzer, M., Blattmann, P., Kuhling, L., Day, P.M., Schiller, J.T., Helenius, A., 2012. Entry of human papillomavirus type 16 by actin-dependent, clathrin- and lipid raft-independent endocytosis. *PLoS pathogens* 8, e1002657.
- Schiffman, M., Doorbar, J., Wentzensen, N., de Sanjose, S., Fakhry, C., Monk, B.J., Stanley, M.A., Franceschi, S., 2016. Carcinogenic human papillomavirus infection. *Nature reviews. Disease primers* 2, 16086.

- Schiller, J.T., Lowy, D.R., 2014. Virus infection and human cancer: an overview. Recent results in cancer research. Fortschritte der Krebsforschung. Progres dans les recherches sur le cancer 193, 1-10.
- Schlegel, R., Phelps, W.C., Zhang, Y.L., Barbosa, M., 1988. Quantitative keratinocyte assay detects two biological activities of human papillomavirus DNA and identifies viral types associated with cervical carcinoma. The EMBO journal 7, 3181-3187.
- Schneider-Gadicke, A., Schwarz, E., 1986. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. The EMBO journal 5, 2285-2292.
- Sekaric, P., Shamanin, V.A., Luo, J., Androphy, E.J., 2007. hAda3 regulates p14ARF-induced p53 acetylation and senescence. Oncogene 26, 6261-6268.
- Selvey, L.A., Dunn, L.A., Tindle, R.W., Park, D.S., Frazer, I.H., 1994. Human papillomavirus (HPV) type 18 E7 protein is a short-lived steroid-inducible phosphoprotein in HPV-transformed cell lines. The Journal of general virology 75 (Pt 7), 1647-1653.
- Shin, M.K., Sage, J., Lambert, P.F., 2012. Inactivating all three rb family pocket proteins is insufficient to initiate cervical cancer. Cancer research 72, 5418-5427.
- Siddiqui-Jain, A., Drygin, D., Streiner, N., Chua, P., Pierre, F., O'Brien, S.E., Bliesath, J., Omori, M., Huser, N., Ho, C., Proffitt, C., Schwaebe, M.K., Ryckman, D.M., Rice, W.G., Anderes, K., 2010. CX-4945, an orally bioavailable selective inhibitor of protein kinase CK2, inhibits prosurvival and angiogenic signaling and exhibits antitumor efficacy. Cancer research 70, 10288-10298.
- Siddiqui, N., Zwetsloot, A.J., Bachmann, A., Roth, D., Hussain, H., Brandt, J., Kaverina, I., Straube, A., 2019. PTPN21 and Hook3 relieve KIF1C autoinhibition and activate intracellular transport. Nature communications 10, 2693.
- Singh, M., Krajewski, M., Mikolajka, A., Holak, T.A., 2005. Molecular determinants for the complex formation between the retinoblastoma protein and LXCXE sequences. The Journal of biological chemistry 280, 37868-37876.
- Singh, R., Kuscu, C., Quinlan, A., Qi, Y., Adli, M., 2015. Cas9-chromatin binding information enables more accurate CRISPR off-target prediction. Nucleic acids research 43, e118.
- Sitz, J., Blanchet, S.A., Gameiro, S.F., Biquand, E., Morgan, T.M., Galloy, M., Dessapt, J., Lavoie, E.G., Blondeau, A., Smith, B.C., Mymryk, J.S., Moody, C.A., Fradet-Turcotte, A., 2019. Human papillomavirus E7

- oncoprotein targets RNF168 to hijack the host DNA damage response. *Proceedings of the National Academy of Sciences*, 201906102.
- Smith-McCune, K., Kalman, D., Robbins, C., Shivakumar, S., Yuschenkoff, L., Bishop, J.M., 1999. Intranuclear localization of human papillomavirus 16 E7 during transformation and preferential binding of E7 to the Rb family member p130. *Proceedings of the National Academy of Sciences of the United States of America* 96, 6999-7004.
- Smotkin, D., Wettstein, F.O., 1986. Transcription of human papillomavirus type 16 early genes in a cervical cancer and a cancer-derived cell line and identification of the E7 protein. *Proceedings of the National Academy of Sciences* 83, 4680-4684.
- Smotkin, D., Wettstein, F.O., 1987. The major human papillomavirus protein in cervical cancers is a cytoplasmic phosphoprotein. *Journal of virology* 61, 1686-1689.
- Solares, A.M., Santana, A., Baladron, I., Valenzuela, C., Gonzalez, C.A., Diaz, A., Castillo, D., Ramos, T., Gomez, R., Alonso, D.F., Herrera, L., Sigman, H., Perea, S.E., Acevedo, B.E., Lopez-Saura, P., 2009. Safety and preliminary efficacy data of a novel casein kinase 2 (CK2) peptide inhibitor administered intralesionally at four dose levels in patients with cervical malignancies. *BMC cancer* 9, 146.
- Song, H., Hu, J., Chen, W., Elliott, G., Andre, P., Gao, B., Yang, Y., 2010. Planar cell polarity breaks bilateral symmetry by controlling ciliary positioning. *Nature* 466, 378-382.
- Songyang, Z., Fanning, A.S., Fu, C., Xu, J., Marfatia, S.M., Chishti, A.H., Crompton, A., Chan, A.C., Anderson, J.M., Cantley, L.C., 1997. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. *Science* 275, 73-77.
- Soto, D.R., Barton, C., Munger, K., McLaughlin-Drubin, M.E., 2017. KDM6A addiction of cervical carcinoma cell lines is triggered by E7 and mediated by p21CIP1 suppression of replication stress. *PLoS pathogens* 13, e1006661.
- Southern, S.A., Lewis, M.H., Herrington, C.S., 2004. Induction of tetrasomy by human papillomavirus type 16 E7 protein is independent of pRb binding and disruption of differentiation. *British journal of cancer* 90, 1949-1954.
- Spangle, J.M., Munger, K., 2010. The human papillomavirus type 16 E6 oncoprotein activates mTORC1 signaling and increases protein synthesis. *Journal of virology* 84, 9398-9407.
- Spangle, J.M., Munger, K., 2013. The HPV16 E6 oncoprotein causes prolonged receptor protein tyrosine kinase signaling and enhances internalization of phosphorylated receptor species. *PLoS pathogens* 9, e1003237.

- Spardy, N., Covella, K., Cha, E., Hoskins, E.E., Wells, S.I., Duensing, A., Duensing, S., 2009. Human papillomavirus 16 E7 oncoprotein attenuates DNA damage checkpoint control by increasing the proteolytic turnover of claspin. *Cancer research* 69, 7022-7029.
- Srivastava, K., Pickard, A., McDade, S., McCance, D.J., 2017. p63 drives invasion in keratinocytes expressing HPV16 E6/E7 genes through regulation of Src-FAK signalling. *Oncotarget* 8, 16202-16219.
- Strutt, H., Searle, E., Thomas-Macarthur, V., Brookfield, R., Strutt, D., 2013. A Cul-3-BTB ubiquitylation pathway regulates junctional levels and asymmetry of core planar polarity proteins. *Development* 140, 1693-1702.
- Strutt, H., Strutt, D., 2008. Differential stability of flamingo protein complexes underlies the establishment of planar polarity. *Current biology : CB* 18, 1555-1564.
- Subbaiah, V.K., Kranjec, C., Thomas, M., Banks, L., 2011. PDZ domains: the building blocks regulating tumorigenesis. *The Biochemical journal* 439, 195-205.
- Suzuki, H., Maruyama, R., Yamamoto, E., Kai, M., 2012. DNA methylation and microRNA dysregulation in cancer. *Molecular oncology* 6, 567-578.
- Szalmas, A., Tomaic, V., Basukala, O., Massimi, P., Mittal, S., Konya, J., Banks, L., 2017. The PTPN14 Tumor Suppressor Is a Degradation Target of Human Papillomavirus E7. *Journal of virology* 91.
- Tan, M.J., White, E.A., Sowa, M.E., Harper, J.W., Aster, J.C., Howley, P.M., 2012. Cutaneous beta-human papillomavirus E6 proteins bind Mastermind-like coactivators and repress Notch signaling. *Proceedings of the National Academy of Sciences of the United States of America* 109, E1473-1480.
- Tanaka, A., Noda, T., Yajima, H., Hatanaka, M., Ito, Y., 1989. Identification of a transforming gene of human papillomavirus type 16. *Journal of virology* 63, 1465-1469.
- Tang, S., Tao, M., McCoy, J.P., Jr., Zheng, Z.M., 2006. The E7 oncoprotein is translated from spliced E6*I transcripts in high-risk human papillomavirus type 16- or type 18-positive cervical cancer cell lines via translation reinitiation. *Journal of virology* 80, 4249-4263.
- Tasaki, T., Mulder, L.C., Iwamatsu, A., Lee, M.J., Davydov, I.V., Varshavsky, A., Muesing, M., Kwon, Y.T., 2005. A family of mammalian E3 ubiquitin ligases that contain the UBR box motif and recognize N-degrons. *Molecular and cellular biology* 25, 7120-7136.
- Thatte, J., Massimi, P., Thomas, M., Boon, S.S., Banks, L., 2018. The HPV E6 PDZ Binding Motif links DNA Damage Response Signaling to E6 Inhibition of p53 Transcriptional Activity. *Journal of virology*.

- Thierry, F., Yaniv, M., 1987. The BPV1-E2 trans-acting protein can be either an activator or a repressor of the HPV18 regulatory region. *The EMBO journal* 6, 3391-3397.
- Thomas, M., Banks, L., 1998. Inhibition of Bak-induced apoptosis by HPV-18 E6. *Oncogene* 17, 2943-2954.
- Thomas, M., Banks, L., 1999. Human papillomavirus (HPV) E6 interactions with Bak are conserved amongst E6 proteins from high and low risk HPV types. *The Journal of general virology* 80 (Pt 6), 1513-1517.
- Thomas, M., Massimi, P., Jenkins, J., Banks, L., 1995. HPV-18 E6 mediated inhibition of p53 DNA binding activity is independent of E6 induced degradation. *Oncogene* 10, 261-268.
- Thomas, M., Myers, M.P., Massimi, P., Guarnaccia, C., Banks, L., 2016. Analysis of Multiple HPV E6 PDZ Interactions Defines Type-Specific PDZ Fingerprints That Predict Oncogenic Potential. *PLoS pathogens* 12, e1005766.
- Thomas, M., Narayan, N., Pim, D., Tomaic, V., Massimi, P., Nagasaka, K., Kranjec, C., Gammoh, N., Banks, L., 2008. Human papillomaviruses, cervical cancer and cell polarity. *Oncogene* 27, 7018-7030.
- Thomas, M.C., Chiang, C.M., 2005. E6 oncoprotein represses p53-dependent gene activation via inhibition of protein acetylation independently of inducing p53 degradation. *Molecular cell* 17, 251-264.
- Tian, R., Li, X., Gao, Y., Li, Y., Yang, P., Wang, K., 2018. Identification and validation of the role of matrix metalloproteinase-1 in cervical cancer. *International journal of oncology* 52, 1198-1208.
- Todorovic, B., Hung, K., Massimi, P., Avvakumov, N., Dick, F.A., Shaw, G.S., Banks, L., Mymryk, J.S., 2012. Conserved region 3 of human papillomavirus 16 E7 contributes to deregulation of the retinoblastoma tumor suppressor. *Journal of virology* 86, 13313-13323.
- Todorovic, B., Massimi, P., Hung, K., Shaw, G.S., Banks, L., Mymryk, J.S., 2011. Systematic analysis of the amino acid residues of human papillomavirus type 16 E7 conserved region 3 involved in dimerization and transformation. *Journal of virology* 85, 10048-10057.
- Tokuchi, H., Higashitsuji, H., Nishiyama, H., Nonoguchi, K., Nagao, T., Xue, J.H., Itoh, K., Ogawa, O., Fujita, J., 1999. Expression of protein tyrosine phosphatase PTP-RL10 and its isoform in the mouse testis. *International journal of urology : official journal of the Japanese Urological Association* 6, 572-577.
- Tomaic, V., Gardiol, D., Massimi, P., Ozbun, M., Myers, M., Banks, L., 2009a. Human and primate tumour viruses use PDZ binding as an evolutionarily conserved mechanism of targeting cell polarity regulators. *Oncogene* 28, 1-8.

- Tomaic, V., Pim, D., Banks, L., 2009b. The stability of the human papillomavirus E6 oncoprotein is E6AP dependent. *Virology* 393, 7-10.
- Tommasino, M., Adamczewski, J.P., Carlotti, F., Barth, C.F., Manetti, R., Contorni, M., Cavalieri, F., Hunt, T., Crawford, L., 1993. HPV16 E7 protein associates with the protein kinase p33CDK2 and cyclin A. *Oncogene* 8, 195-202.
- Tonks, N.K., 2006. Protein tyrosine phosphatases: from genes, to function, to disease. *Nature reviews. Molecular cell biology* 7, 833-846.
- Tonks, N.K., 2013. Protein tyrosine phosphatases--from housekeeping enzymes to master regulators of signal transduction. *The FEBS journal* 280, 346-378.
- Tonks, N.K., Diltz, C.D., Fischer, E.H., 1988. Characterization of the major protein-tyrosine-phosphatases of human placenta. *The Journal of biological chemistry* 263, 6731-6737.
- Torban, E., Patenaude, A.M., Leclerc, S., Rakowiecki, S., Gauthier, S., Andelfinger, G., Epstein, D.J., Gros, P., 2008. Genetic interaction between members of the Vangl family causes neural tube defects in mice. *Proceedings of the National Academy of Sciences of the United States of America* 105, 3449-3454.
- Tree, D.R., Shulman, J.M., Rousset, R., Scott, M.P., Gubb, D., Axelrod, J.D., 2002. Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. *Cell* 109, 371-381.
- Tugizov, S., Berline, J., Herrera, R., Penaranda, M.E., Nakagawa, M., Palefsky, J., 2005. Inhibition of human papillomavirus type 16 E7 phosphorylation by the S100 MRP-8/14 protein complex. *Journal of virology* 79, 1099-1112.
- Ueno, K., Hiura, M., Suehiro, Y., Hazama, S., Hirata, H., Oka, M., Imai, K., Dahiya, R., Hinoda, Y., 2008. Frizzled-7 as a potential therapeutic target in colorectal cancer. *Neoplasia* 10, 697-705.
- UK., I.A.f.R.o.C.a.C.R., 2012. World Cancer Factsheet. Cancer Research UK, London, 2012.
- Uranbileg, B., Yamamoto, H., Park, J.H., Mohanty, A.R., Arakawa-Takeuchi, S., Jinno, S., Okayama, H., 2012. Cdc6 protein activates p27KIP1-bound Cdk2 protein only after the bound p27 protein undergoes C-terminal phosphorylation. *The Journal of biological chemistry* 287, 6275-6283.
- Uysal-Onganer, P., Kawano, Y., Caro, M., Walker, M.M., Diez, S., Darrington, R.S., Waxman, J., Kypta, R.M., 2010. Wnt-11 promotes neuroendocrine-like differentiation, survival and migration of prostate cancer cells. *Molecular cancer* 9, 55.

- Valdovinos-Torres, H., Orozco-Morales, M., Pedroza-Saavedra, A., Padilla-Noriega, L., Esquivel-Guadarrama, F., Gutierrez-Xicotencatl, L., 2008. Different Isoforms of HPV-16 E7 Protein are Present in Cytoplasm and Nucleus. *The open virology journal* 2, 15-23.
- Vande Pol, S.B., Klingelutz, A.J., 2013. Papillomavirus E6 oncoproteins. *Virology* 445, 115-137.
- VanderVorst, K., Hatakeyama, J., Berg, A., Lee, H., Carraway, K.L., 3rd, 2018. Cellular and molecular mechanisms underlying planar cell polarity pathway contributions to cancer malignancy. *Seminars in cell & developmental biology* 81, 78-87.
- Venuti, A., Paolini, F., Nasir, L., Corteggio, A., Roperto, S., Campo, M.S., Borzacchiello, G., 2011. Papillomavirus E5: the smallest oncoprotein with many functions. *Molecular cancer* 10, 140.
- Vliet-Gregg, P.A., Hamilton, J.R., Katzenellenbogen, R.A., 2013. NFX1-123 and human papillomavirus 16E6 increase Notch expression in keratinocytes. *Journal of virology* 87, 13741-13750.
- Vogt, M., Butz, K., Dymalla, S., Semzow, J., Hoppe-Seyler, F., 2006. Inhibition of Bax activity is crucial for the antiapoptotic function of the human papillomavirus E6 oncoprotein. *Oncogene* 25, 4009-4015.
- Vousden, K.H., Doniger, J., DiPaolo, J.A., Lowy, D.R., 1988. The E7 open reading frame of human papillomavirus type 16 encodes a transforming gene. *Oncogene research* 3, 167-175.
- Wadham, C., Gamble, J.R., Vadas, M.A., Khew-Goodall, Y., 2003. The protein tyrosine phosphatase Pez is a major phosphatase of adherens junctions and dephosphorylates beta-catenin. *Molecular biology of the cell* 14, 2520-2529.
- Waggoner, S.E., Woodworth, C.D., Stoler, M.H., Barnes, W.A., Delgado, G., DiPaolo, J.A., 1990. Human cervical cells immortalized in vitro with oncogenic human papillomavirus DNA differentiate dysplastically in vivo. *Gynecologic oncology* 38, 407-412.
- Wald, J.H., Hatakeyama, J., Printsev, I., Cuevas, A., Fry, W.H.D., Saldana, M.J., VanderVorst, K., Rowson-Hodel, A., Angelastro, J.M., Sweeney, C., Carraway, K.L.R., 2017a. Suppression of planar cell polarity signaling and migration in glioblastoma by Nrdp1-mediated Dvl polyubiquitination. *Oncogene* 36, 5158-5167.
- Wald, J.H., Hatakeyama, J., Printsev, I., Cuevas, A., Fry, W.H.D., Saldana, M.J., VanderVorst, K., Rowson-Hodel, A., Angelastro, J.M., Sweeney, C., Carraway Rd, K.L., 2017b. Suppression of planar cell polarity signaling and migration in glioblastoma by Nrdp1-mediated Dvl polyubiquitination. *Oncogene*.

- Wallace, N.A., Galloway, D.A., 2014. Manipulation of cellular DNA damage repair machinery facilitates propagation of human papillomaviruses. *Seminars in cancer biology* 26, 30-42.
- Wallace, N.A., Khanal, S., Robinson, K.L., Wendel, S.O., Messer, J.J., Galloway, D.A., 2017. High-Risk Alphapapillomavirus Oncogenes Impair the Homologous Recombination Pathway. *Journal of virology* 91.
- Wang, H., Zhang, X., Huang, L., Li, J., Qu, S., Pan, F., 2014. Matrix metalloproteinase-14 expression and its prognostic value in cervical carcinoma. *Cell biochemistry and biophysics* 70, 729-734.
- Wang, J., Sampath, A., Raychaudhuri, P., Bagchi, S., 2001. Both Rb and E7 are regulated by the ubiquitin proteasome pathway in HPV-containing cervical tumor cells. *Oncogene* 20, 4740-4749.
- Wang, W., Huang, J., Wang, X., Yuan, J., Li, X., Feng, L., Park, J.I., Chen, J., 2012. PTPN14 is required for the density-dependent control of YAP1. *Genes & development* 26, 1959-1971.
- Wang, Y., Guo, N., Nathans, J., 2006. The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26, 2147-2156.
- Wang, Z., Shen, D., Parsons, D.W., Bardelli, A., Sager, J., Szabo, S., Ptak, J., Silliman, N., Peters, B.A., van der Heijden, M.S., Parmigiani, G., Yan, H., Wang, T.L., Riggins, G., Powell, S.M., Willson, J.K., Markowitz, S., Kinzler, K.W., Vogelstein, B., Velculescu, V.E., 2004. Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science* 304, 1164-1166.
- Wansleebe, C., Feitsma, H., Montcouquiol, M., Kroon, C., Cuppen, E., Meijlink, F., 2010. Planar cell polarity defects and defective Vangl2 trafficking in mutants for the COPII gene Sec24b. *Development* 137, 1067-1073.
- Warrington, S.J., Strutt, H., Fisher, K.H., Strutt, D., 2017. A Dual Function for Prickle in Regulating Frizzled Stability during Feedback-Dependent Amplification of Planar Polarity. *Current biology : CB* 27, 2784-2797 e2783.
- Watanabe, S., Yoshiike, K., 1988. Transformation of rat 3Y1 cells by human papillomavirus type-18 DNA. *International journal of cancer. Journal international du cancer* 41, 896-900.
- Weijzen, S., Zlobin, A., Braid, M., Miele, L., Kast, W.M., 2003. HPV16 E6 and E7 oncoproteins regulate Notch-1 expression and cooperate to induce transformation. *Journal of cellular physiology* 194, 356-362.
- Werness, B.A., Levine, A.J., Howley, P.M., 1990. Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 248, 76-79.

- White, E.A., Howley, P.M., 2013. Proteomic approaches to the study of papillomavirus-host interactions. *Virology* 435, 57-69.
- White, E.A., Munger, K., Howley, P.M., 2016. High-Risk Human Papillomavirus E7 Proteins Target PTPN14 for Degradation. *mBio* 7.
- White, E.A., Sowa, M.E., Tan, M.J., Jeudy, S., Hayes, S.D., Santha, S., Munger, K., Harper, J.W., Howley, P.M., 2012. Systematic identification of interactions between host cell proteins and E7 oncoproteins from diverse human papillomaviruses. *Proceedings of the National Academy of Sciences of the United States of America* 109, E260-267.
- WHO, 2013a. WHO guidance note. Comprehensive cervical cancer prevention and control: a healthier future for girls and women. World Health Organization, Geneva.
- WHO, 2013b. WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. World Health Organization, Geneva.
- WHO, 2017a. Human papillomavirus (HPV).
- WHO, 2017b. Human papillomavirus vaccines: WHO position paper, May 2017. *Weekly epidemiological record* 92, 241–268
- Wigler, M., Sweet, R., Sim, G.K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S., Axel, R., 1979. Transformation of mammalian cells with genes from procaryotes and eucaryotes. *Cell* 16, 777-785.
- Williams, B.B., Cantrell, V.A., Mundell, N.A., Bennett, A.C., Quick, R.E., Jessen, J.R., 2012. VANG2 regulates membrane trafficking of MMP14 to control cell polarity and migration. *Journal of cell science* 125, 2141-2147.
- Wilson, K.E., Li, Y.W., Yang, N., Shen, H., Orillion, A.R., Zhang, J., 2014. PTPN14 forms a complex with Kibra and LATS1 proteins and negatively regulates the YAP oncogenic function. *The Journal of biological chemistry* 289, 23693-23700.
- Wirkner, U., Voss, H., Lichter, P., Weitz, S., Ansorge, W., Pyerin, W., 1992. Human casein kinase II subunit alpha: sequence of a processed (pseudo)gene and its localization on chromosome 11. *Biochimica et biophysica acta* 1131, 220-222.
- Wise-Draper, T.M., Allen, H.V., Thobe, M.N., Jones, E.E., Habash, K.B., Munger, K., Wells, S.I., 2005. The human DEK proto-oncogene is a senescence inhibitor and an upregulated target of high-risk human papillomavirus E7. *Journal of virology* 79, 14309-14317.
- Wolff, T., Rubin, G.M., 1998. Strabismus, a novel gene that regulates tissue polarity and cell fate decisions in *Drosophila*. *Development* 125, 1149-1159.

- Woodworth, C.D., Bowden, P.E., Doniger, J., Pirisi, L., Barnes, W., Lancaster, W.D., DiPaolo, J.A., 1988. Characterization of normal human exocervical epithelial cells immortalized in vitro by papillomavirus types 16 and 18 DNA. *Cancer research* 48, 4620-4628.
- Woodworth, C.D., Doniger, J., DiPaolo, J.A., 1989. Immortalization of human foreskin keratinocytes by various human papillomavirus DNAs corresponds to their association with cervical carcinoma. *Journal of virology* 63, 159-164.
- Wu, E.W., Clemens, K.E., Heck, D.V., Munger, K., 1993. The human papillomavirus E7 oncoprotein and the cellular transcription factor E2F bind to separate sites on the retinoblastoma tumor suppressor protein. *Journal of virology* 67, 2402-2407.
- Wu, J., Mlodzik, M., 2008. The frizzled extracellular domain is a ligand for Van Gogh/Stbm during nonautonomous planar cell polarity signaling. *Developmental cell* 15, 462-469.
- Wu, Z.Z., Lu, H.P., Chao, C.C., 2010. Identification and functional analysis of genes which confer resistance to cisplatin in tumor cells. *Biochemical pharmacology* 80, 262-276.
- Xu, M., Katzenellenbogen, R.A., Grandori, C., Galloway, D.A., 2013. An unbiased in vivo screen reveals multiple transcription factors that control HPV E6-regulated hTERT in keratinocytes. *Virology* 446, 17-24.
- Yablonska, S., Hoskins, E.E., Wells, S.I., Khan, S.A., 2013. Identification of miRNAs dysregulated in human foreskin keratinocytes (HFKs) expressing the human papillomavirus (HPV) Type 16 E6 and E7 oncoproteins. *Microna* 2, 2-13.
- Yadav, L., Puri, N., Rastogi, V., Satpute, P., Ahmad, R., Kaur, G., 2014. Matrix metalloproteinases and cancer - roles in threat and therapy. *Asian Pacific journal of cancer prevention : APJCP* 15, 1085-1091.
- Yagyu, R., Hamamoto, R., Furukawa, Y., Okabe, H., Yamamura, T., Nakamura, Y., 2002. Isolation and characterization of a novel human gene, VANG1, as a therapeutic target for hepatocellular carcinoma. *International journal of oncology* 20, 1173-1178.
- Yamato, K., Yamada, T., Kizaki, M., Ui-Tei, K., Natori, Y., Fujino, M., Nishihara, T., Ikeda, Y., Nasu, Y., Saigo, K., Yoshinouchi, M., 2008. New highly potent and specific E6 and E7 siRNAs for treatment of HPV16 positive cervical cancer. *Cancer gene therapy* 15, 140-153.
- Yan, H., Xin, S., Wang, H., Ma, J., Zhang, H., Wei, H., 2015. Baicalein inhibits MMP-2 expression in human ovarian cancer cells by suppressing the p38 MAPK-dependent NF-kappaB signaling pathway. *Anticancer Drugs* 26, 649-656.

- Yang, L., Guell, M., Byrne, S., Yang, J.L., De Los Angeles, A., Mali, P., Aach, J., Kim-Kiselak, C., Briggs, A.W., Rios, X., Huang, P.Y., Daley, G., Church, G., 2013. Optimization of scarless human stem cell genome editing. *Nucleic acids research* 41, 9049-9061.
- Yasumoto, S., Burkhardt, A.L., Doniger, J., DiPaolo, J.A., 1986. Human papillomavirus type 16 DNA-induced malignant transformation of NIH 3T3 cells. *Journal of virology* 57, 572-577.
- Yoshinouchi, M., Yamada, T., Kizaki, M., Fen, J., Koseki, T., Ikeda, Y., Nishihara, T., Yamato, K., 2003. In vitro and in vivo growth suppression of human papillomavirus 16-positive cervical cancer cells by E6 siRNA. *Molecular therapy : the journal of the American Society of Gene Therapy* 8, 762-768.
- Young, L.C., Hartig, N., Munoz-Alegre, M., Oses-Prieto, J.A., Durdu, S., Bender, S., Vijayakumar, V., Vietri Rudan, M., Gewinner, C., Henderson, S., Jathoul, A.P., Ghatrora, R., Lythgoe, M.F., Burlingame, A.L., Rodriguez-Viciana, P., 2013. An MRAS, SHOC2, and SCRIB complex coordinates ERK pathway activation with polarity and tumorigenic growth. *Molecular cell* 52, 679-692.
- Yun, H.Y., Kim, M.W., Lee, H.S., Kim, W., Shin, J.H., Kim, H., Shin, H.C., Park, H., Oh, B.H., Kim, W.K., Bae, K.H., Lee, S.C., Lee, E.W., Ku, B., Kim, S.J., 2019. Structural basis for recognition of the tumor suppressor protein PTPN14 by the oncoprotein E7 of human papillomavirus. *PLoS biology* 17, e3000367.
- Yutsudo, M., Okamoto, Y., Hakura, A., 1988. Functional dissociation of transforming genes of human papillomavirus type 16. *Virology* 166, 594-597.
- Zanier, K., Charbonnier, S., Sidi, A.O., McEwen, A.G., Ferrario, M.G., Poussin-Courmontagne, P., Cura, V., Brimer, N., Babah, K.O., Ansari, T., Muller, I., Stote, R.H., Cavarelli, J., Vande Pol, S., Trave, G., 2013. Structural basis for hijacking of cellular LxxLL motifs by papillomavirus E6 oncoproteins. *Science* 339, 694-698.
- Zanier, K., ould M'hamed ould Sidi, A., Boulade-Ladame, C., Rybin, V., Chappelle, A., Atkinson, A., Kieffer, B., Trave, G., 2012. Solution structure analysis of the HPV16 E6 oncoprotein reveals a self-association mechanism required for E6-mediated degradation of p53. *Structure* 20, 604-617.
- Zeng, Z., Zhou, W., Duan, L., Zhang, J., Lu, X., Jin, L., Yu, Y., 2019. Circular RNA circ-VANGL1 as a competing endogenous RNA contributes to bladder cancer progression by regulating miR-605-3p/VANGL1 pathway. *Journal of cellular physiology* 234, 3887-3896.
- Zerfass-Thome, K., Zwerschke, W., Mannhardt, B., Tindle, R., Botz, J.W., Jansen-Durr, P., 1996. Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. *Oncogene* 13, 2323-2330.

- Zerfass, K., Schulze, A., Spitkovsky, D., Friedman, V., Henglein, B., Jansen-Durr, P., 1995. Sequential activation of cyclin E and cyclin A gene expression by human papillomavirus type 16 E7 through sequences necessary for transformation. *Journal of virology* 69, 6389-6399.
- Zhai, Y., Hotary, K.B., Nan, B., Bosch, F.X., Munoz, N., Weiss, S.J., Cho, K.R., 2005. Expression of membrane type 1 matrix metalloproteinase is associated with cervical carcinoma progression and invasion. *Cancer research* 65, 6543-6550.
- Zhang, B., Chen, W., Roman, A., 2006. The E7 proteins of low- and high-risk human papillomaviruses share the ability to target the pRB family member p130 for degradation. *Proceedings of the National Academy of Sciences of the United States of America* 103, 437-442.
- Zhang, J.G., Farley, A., Nicholson, S.E., Willson, T.A., Zugaro, L.M., Simpson, R.J., Moritz, R.L., Cary, D., Richardson, R., Hausmann, G., Kile, B.T., Kent, S.B., Alexander, W.S., Metcalf, D., Hilton, D.J., Nicola, N.A., Baca, M., 1999. The conserved SOCS box motif in suppressors of cytokine signaling binds to elongins B and C and may couple bound proteins to proteasomal degradation. *Proceedings of the National Academy of Sciences of the United States of America* 96, 2071-2076.
- Zhang, P., Guo, A., Possemato, A., Wang, C., Beard, L., Carlin, C., Markowitz, S.D., Polakiewicz, R.D., Wang, Z., 2013. Identification and functional characterization of p130Cas as a substrate of protein tyrosine phosphatase nonreceptor 14. *Oncogene* 32, 2087-2095.
- Zhao, J., Lee, E.E., Kim, J., Yang, R., Chamseddin, B., Ni, C., Gusho, E., Xie, Y., Chiang, C.M., Buszczak, M., Zhan, X., Laimins, L., Wang, R.C., 2019. Transforming activity of an oncoprotein-encoding circular RNA from human papillomavirus. *Nature communications* 10, 2300.
- Zheng, L., Ding, H., Lu, Z., Li, Y., Pan, Y., Ning, T., Ke, Y., 2008. E3 ubiquitin ligase E6AP-mediated TSC2 turnover in the presence and absence of HPV16 E6. *Genes to Cells* 13, 285-294.
- Zhou, X., Munger, K., 2009. Expression of the human papillomavirus type 16 E7 oncoprotein induces an autophagy-related process and sensitizes normal human keratinocytes to cell death in response to growth factor deprivation. *Virology* 385, 192-197.
- Zhou, X., Spangle, J.M., Munger, K., 2009. Expression of a viral oncoprotein in normal human epithelial cells triggers an autophagy-related process: is autophagy an "Achilles' heel" of human cancers? *Autophagy* 5, 578-579.
- Zhu, D., Ye, M., Zhang, W., 2015. E6/E7 oncoproteins of high risk HPV-16 upregulate MT1-MMP, MMP-2 and MMP-9 and promote the migration of cervical cancer cells. *International journal of clinical and experimental pathology* 8, 4981-4989.

- Zimmermann, H., Degenkolbe, R., Bernard, H.U., O'Connor, M.J., 1999. The human papillomavirus type 16 E6 oncoprotein can down-regulate p53 activity by targeting the transcriptional coactivator CBP/p300. *Journal of virology* 73, 6209-6219.
- Zine El Abidine, A., Tomaic, V., Bel Haj Rhouma, R., Massimi, P., Guizani, I., Boubaker, S., Ennaifer, E., Banks, L., 2017. A naturally occurring variant of HPV-16 E7 exerts increased transforming activity through acquisition of an additional phospho-acceptor site. *Virology* 500, 218-225.
- Zwerschke, W., Mazurek, S., Massimi, P., Banks, L., Eigenbrodt, E., Jansen-Durr, P., 1999. Modulation of type M2 pyruvate kinase activity by the human papillomavirus type 16 E7 oncoprotein. *Proceedings of the National Academy of Sciences of the United States of America* 96, 1291-1296.